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DATE: Thursday, January 04, 2007

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	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=ADJ		
☐	L8	therapeutic domain and anchoring domain	4
☐	L7	therapeutic domain and activity and anchoring	6
	DB=USPT; PLUR=YES; OP=ADJ		
☐	L6	L3 and activity	1
☐	L5	L3 and extracellular	0
☐	L4	L3 and extracellular activity	0
☐	L3	L2 and therapeutic	1
☐	L2	L1 and anchoring	1
☐	L1	6503703	1

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=> file medline hcaplus biosis bitechds scisearch
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FILE 'SCISEARCH' ENTERED AT 18:00:23 ON 04 JAN 2007
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=> s therapeutic domain and anchoring domain
L1            3 THERAPEUTIC DOMAIN AND ANCHORING DOMAIN

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2            2 DUP REM L1 (1 DUPLICATE REMOVED)

=> d l2 1-2 ibib ab

L2   ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         2005:450844  HCAPLUS
DOCUMENT NUMBER:          143:1221
TITLE:                    Antiviral proteins blocking infection using
                          glycosaminoglycan-binding domains to bind protease
                          inhibitors or sialidases to cell surfaces for
                          treatment and preventing influenza
INVENTOR(S):              Fang, Fang; Malakhov, Michael
PATENT ASSIGNEE(S):       USA
SOURCE:                   U.S. Pat. Appl. Publ., 82 pp., Cont.-in-part of U.S.
                          Ser. No. 718,986.
                          CODEN: USXXCO
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   2
PATENT INFORMATION:

     PATENT NO.            KIND   DATE        APPLICATION NO.          DATE
     ----------------      ----   --------    --------------------     --------
     US 2005112751          A1    20050526    US 2004-939262           20040910
     US 2005004020          A1    20050106    US 2003-718986           20031121
     WO 2006031291          A2    20060323    WO 2005-US25831          20050721
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ,
             LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA,
             NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK,
             SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU,
             ZA, ZM, ZW
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RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

PRIORITY APPLN. INFO.:		
US 2002-428535P	P	20021122
US 2003-464217P	P	20030419
US 2003-718986	A2	20031121
US 2004-561749P	P	20040413
US 2004-580084P	P	20040616
US 2004-939262	A	20040910

AB Fusion proteins that use a glycosaminoglycan-binding domain to bind antibacterial proteins to a cell surface are described for the treatment of microbial infection, esp. influenza. Use of the glycosaminoglycan-binding domains targets the protein to the surface of epithelial cells, and this binds the therapeutic domain to the cell surface to prevent infection of the target cell by a pathogen such as an influenza virus. The glycosaminoglycan-binding anchoring domain may be from a mammalian protein, such as human platelet factor 4, interleukin 8, antithrombin III, or apolipoprotein E. The therapeutic domain may be an enzyme, such as a sialidase, or a protease inhibitor for a host enzyme involved in processing a viral protein. Examples of protease inhibitors are aprotinin, leupeptin, soybean proteinase inhibitor, e-aminocaproic acid, or n-p-tosyl-L-lysine.

L2 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 1

ACCESSION NUMBER:	2004:470946 HCAPLUS
DOCUMENT NUMBER:	141:33763
TITLE:	Broad spectrum antivirals comprising a target cell-anchoring GAG-binding domain fused with protease inhibitor or sialidase, for treatment and preventing influenza
INVENTOR(S):	Yu, Mang; Fang, Fang
PATENT ASSIGNEE(S):	USA
SOURCE:	PCT Int. Appl., 75 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004047735	A2	20040610	WO 2003-US37158	20031121
WO 2004047735	A3	20040923		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2506526	A1	20040610	CA 2003-2506526	20031121
AU 2003294401	A1	20040618	AU 2003-294401	20031121
EP 1567185	A2	20050831	EP 2003-789884	20031121
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
CN 1729013	A	20060201	CN 2003-80107241	20031121
JP 2006508193	T	20060309	JP 2005-510377	20031121

PRIORITY APPLN. INFO.:		
US 2002-428535P	P	20021122
US 2003-464217P	P	20030419

WO 2003-US37158 W 20031121

AB The present invention provides new protein-based compns. and methods for preventing and treating pathogen infection, particularly influenza. The compds. have at least one N-terminal or C-terminal anchoring domain that anchors the compd. to the surface of a target epithelial cell, and at least one therapeutic domain that can act extracellularly to prevent infection of the target cell by a pathogen, such as a influenza virus. The said anchoring domain comprises a GAG-binding motif from a mammalian protein, such as human platelet factor 4, interleukin 8, antithrombin III, apolipoprotein E, angio-assocd. cell migratory protein (AAMP), or amphiregulin. The said therapeutic domain comprises enzyme, such as sialidase, or protease inhibitor for host enzyme involved in processing a viral protein. Examples of protease inhibitors are aprotinin, leupeptin, soybean proteinase inhibitor, e-aminocaproic acid, or n-p-tosyl-L-lysine.

=> s reportor domain and anchoring domain
L3 0 REPORTOR DOMAIN AND ANCHORING DOMAIN

=> s reportor and anchoring domain
L4 0 REPORTOR AND ANCHORING DOMAIN

=> s therapeutic and extracellular and anchoring domain
L5 3 THERAPEUTIC AND EXTRACELLULAR AND ANCHORING DOMAIN

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 3 DUP REM L5 (0 DUPLICATES REMOVED)

=> d l6 1-3 ibib ab

L6 ANSWER 1 OF 3 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2006-27540 BIOTECHDS
TITLE: Novel isolated receptor for advanced glycation endproducts isoform polypeptide being intron fusion protein, useful for treating cancer, diabetes, multiple sclerosis, autoimmune disease and neurodegenerative disease;
vector-mediated gene transfer and expression in human cell for use in disease gene therapy and cell therapy
AUTHOR: JIN P; SHEPARD H M
PATENT ASSIGNEE: RECEPTOR BIOLOGIX INC
PATENT INFO: WO 2006119510 9 Nov 2006
APPLICATION INFO: WO 2006-US17786 4 May 2006
PRIORITY INFO: US 2005-736134 10 Nov 2005; US 2005-678076 4 May 2005
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2006-767325 [78]
AB DERWENT ABSTRACT:
NOVELTY - An isolated receptor for advanced glycation endproducts (RAGE) isoform polypeptide (P1), is new. The RAGE polypeptide is an intron fusion protein encoded by a sequence of nucleotides that includes all or a portion of an intron chosen from introns 2, 3, 5 and 8 of a cognate RAGE gene.

DETAILED DESCRIPTION - An isolated receptor for advanced glycation endproducts (RAGE) isoform polypeptide (P1), (a) is an intron fusion protein encoded by a sequence of nucleotides that includes all or a portion of an intron chosen from introns 2, 3, 5 and 8 of a cognate RAGE gene, (b) comprising a deletion and/or insertion of one or more amino acids of the first C-type Ig-like domain of RAGE, a deletion and/or insertion of one or more amino acids of the second C-type Ig- like domain of Rage, a deletion and/or insertion of one or more amino acids of the transmembrane domain of RAGE, where the membrane localization of the RAGE isoform is reduced or abolished compared to RAGE, and the RAGE isoform is

an intron fusion protein, or (c) comprises a sequence of amino acids chosen from a sequence that comprises at least 70% sequence identity to a fully defined 146 amino acid (SEQ ID No. 10) sequence given in the specification, a sequence that comprises at least 75% sequence identity to a fully defined 172 amino acid (SEQ ID No. 11) sequence given in the specification, a sequence that comprises at least 86% sequence identity to a fully defined 387 amino acid (SEQ ID No. 12) sequence given in the specification, a sequence that comprises at least 90% sequence identity to a fully defined 266 amino acid (SEQ ID No. 13) sequence given in the specification, and a sequence that comprises at least 93% sequence identity to a fully defined 173 amino acid (SEQ ID No. 14) sequence given in the specification, where sequence identity is compared along the full length of each SEQ ID to the full length sequence of the RAGE isoforms. INDEPENDENT CLAIMS are included for: (1) a RAGE isoform polypeptide that has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more sequence identity to (P1); (2) a nucleic acid molecule encoding (P1); (3) a vector comprising the nucleic acid molecule; (4) a cell comprising the vector or the nucleic acid molecule; (5) a pharmaceutical composition comprising a RAGE isoform or the vector or the nucleic acid molecule; (6) treating a disease or condition, by administering the pharmaceutical composition or the cell to a subject (7) inhibiting tumor invasion or metastasis of a tumor, by administering the composition; (8) a conjugate comprising a RAGE isoform or its active fragment or (P1); (9) a multimeric compound, comprising two or more conjugates comprising a RAGE isoform or its active fragment, or (P1) and one or more other cell surface receptor isoforms or active its fragments, where the resulting compound modulates the activity of a RAGE and/or a cell surface receptor (CSR); (10) a chimeric polypeptide comprising all or at least one domain of a RAGE isoform or its active fragment and all of or at least one domain of a different RAGE isoform or of another cell surface receptor isoform or of a ligand isoform or portion of an isoform that possesses an activity; (11) a pharmaceutical composition comprising a polypeptide or the conjugate or the multimeric compound; (12) treating a disease or condition, by administering the pharmaceutical composition, where the disease or condition is mediated by or involves a CSR in its etiology; (13) a combination comprising one or more RAGE isoform(s) and one or more other CSR isoforms and/or a therapeutic drug; (14) treating a subject by administering the components of the combination, where each component is administered separately, simultaneously, intermittently, in a single composition or their combinations; (15) a polypeptide comprising a domain of a RAGE or a RAGE isoform or its active fragment linked directly or indirectly to serum albumin or other mucin; and (16) use of a RAGE polypeptide or the nucleic acid molecule or the cell or the multimeric compound or the conjugate for the formulation of a medicament for treatment of a disease or condition chosen from diabetes, diabetes-related conditions, cancers, inflammatory diseases, angiogenesis-related conditions, cell proliferation-related conditions, immune disorders, kidney disease, ocular disease, endometriosis, periodontal disease and neurodegenerative diseases.

BIOTECHNOLOGY - Preparation (Disclosed): The RAGE polypeptide is produced by recombinant techniques. Preferred Polypeptide: The sequence of the cognate RAGE gene comprises a fully defined 4557 base pair (SEQ ID No. 325) sequence given in the specification, or is an allelic or its species variant. The isoform comprises a sequence of amino acids chosen from SEQ ID No. 10-12 or is an allelic or its species variant or chosen from SEQ ID No. 10-14 or is an allelic or its species variant. The sequence identity is compared with a mature isoform that lacks a signal sequence or with a precursor form that includes a signal sequence. The RAGE isoform polypeptide is encoded by a nucleic acid molecule that comprises at least one codon from an intron, where the intron is from a gene encoding RAGE. A RAGE isoform polypeptide lacking the signal peptide. The isoform comprises a signal peptide. The allelic variant comprises variations that correspond to one or more of the allelic variations having a fully defined 404 amino acid (SEQ ID No. 4) sequence given in the specification. The RAGE isoform polypeptide contains the

same number of amino acids as in SEQ ID No. 10-14, or the same number but lacking the signal sequence in each. A RAGE isoform polypeptide encoded by a sequence of nucleotides having a fully defined 928, 941, 1165, 994 and 1415 base pair (SEQ ID No. 5-9) sequence given in the specification or an allelic or its species variant. The allelic variant comprises variations that correspond to one or more nucleotides of the allelic variations having a fully defined 1414 base pair (SEQ ID No. 3) sequence given in the specification. The isoform modulates a function or activity of a RAGE receptor. The activity of a RAGE modulated by the polypeptide is chosen from ligand binding, competition with RAGE for ligand binding, ligand endocytosis, regulation of gene expression, signal transduction, interaction with a signal transduction molecule, membrane association and membrane localization. The cell surface receptor isoform is an intron fusion protein. The chimeric polypeptide comprises all of or at least one domain of a RAGE isoform and an intron-encoded portion of a cell surface receptor isoform. Preferred Composition: The composition comprises an amount of the isoform effective for modulating an activity of a cell surface receptor. The isoform modulates a function or activity of a RAGE. The modulation is an inhibition of activity. The isoform of the composition complexes with a RAGE. The composition contains a polypeptide that inhibits angiogenesis, cell proliferation, cell migration, tumor cell growth or tumor cell metastasis. Preferred Nucleic Acid: The nucleic acid molecule, comprises an intron and an exon, where the intron contains a stop codon, the nucleic acid molecule encodes an open reading frame that spans an exon intron junction, and the open reading frame terminates at the stop codon in the intron. The intron encodes one or more amino acids of the encoded RAGE isoform. The stop codon is the first codon in the intron. The isolated nucleic acid molecule comprises a sequence of nucleotides having SEQ ID No. 5-9 or an allelic or its species variant. Preferred Vector: The vector is a mammalian vector, or viral vector. The vector is episomal or that integrates into the chromosome of the cell which it is introduced. Preferred Conjugate: The conjugate comprises a RAGE isoform or its fragment linked to a multimerization domain, where the multimerization domain is chosen from Fc region, leucine zipper, an amino acid sequence comprising a protuberance complementary to an amino acid sequence comprising a hole, hydrophobic domain, hydrophilic domain, an amino acid sequence comprising a free thiol moiety, which reacts to form an intermolecular disulfide bond with a multimerization domain of an additional amino acid sequence, and a protein interaction domain chosen from R subunit of a PKA and an anchoring domain (AD).
The conjugate comprises a RAGE isoform or its domain or functional portion, and a second portion from a different RAGE isoform or from another CSR; one of the portions is all or part of an extracellular domain of an isoform; and the portions are linked directly or through a linker. The CSR is a receptor tyrosine kinase. One portion is from a herstatin polypeptide. Preferred Multimeric Compound: The multimeric compound is a homodimer, heterodimer or a trimer. The multimeric compound comprises a RAGE isoform or domain or its ligand binding portion and a cell surface receptor isoform or its domain or a ligand binding portion or ligand isoform. Preferred Combination: The isoforms and/or drugs are in separate compositions or in a single composition. The CSR isoform is an isoform of an ErbB, VEGFR, FGFR, TNFR, PDGFR, MET, Tie-2 or an EPHA2. The RAGE or RAGE isoform and/or the CSR isoform or other isoform is an extracellular domain or a portion thereof that possesses ligand binding activity or dimerization activity or other activity of a RAGE or CSR. Preferred Method: The composition comprises a nucleic acid molecule or a vector, and the method involves introducing the composition into a cell(s) that have been removed from a host animal, and introducing the cells into the same animal or into an animal compatible with the animal from whom the cells were removed or an animal that has been treated to be compatible. The animal is a human.

ACTIVITY - Antidiabetic; Cytostatic; Antiinflammatory; Antiangiogenic; Nephrotropic; Ophthalmological; Gynecological; Neuroprotective; Antiarthritic; Antirheumatic; Nootropic; Anticonvulsant;

Antiarteriosclerotic; Immunomodulator; Immunosuppressive; Vulnerary; CNS-Gen.; Respiratory-Gen.; Gastrointestinal-Gen. No biological data given.

MECHANISM OF ACTION - RAGE modulator; Gene therapy; Cell therapy.

USE - The RAGE polypeptide, nucleic acid molecule, cell, multimeric compound, or conjugate is useful for treating diabetes, its related conditions, cancers, inflammatory diseases, angiogenesis-related conditions, cell proliferation-related conditions, immune disorders, kidney disease, ocular disease, endometriosis, periodontal disease and neurodegenerative diseases. The RAGE polypeptide is useful for treating rheumatoid arthritis, arthritis, multiple sclerosis, Alzheimer's disease, Creutzfeldt-Jakob disease, Huntington's disease, posterior intraocular inflammation, uveitic disorders, ocular surface inflammatory disorders, macular degeneration, neovascular disease, proliferative vitreoretinopathy, atherosclerosis, type I diabetes, multiple sclerosis, chronic kidney disease. The diabetes-associated condition is chosen from periodontal disease, autoimmune disease, vascular disease associated with wound healing, tubulointerstitial disease, atherosclerosis. The cancer is chosen from carcinoma, lymphoma, blastoma, sarcoma, leukemia, lymphoid malignancies, squamous cell cancer, lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial/uterine carcinoma, salivary gland carcinoma, renal, prostate, vulval, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, head and neck cancer. The disease is an angiogenesis-related disease. The disease is chosen from inflammatory, immune disorders, diabetic retinopathies and/or neuropathies and other inflammatory vascular complications of diabetes, autoimmune diseases, including autoimmune diabetes, atherosclerosis, Crohn's disease, diabetic kidney disease, cystic fibrosis, endometriosis, diabetes-induced vascular injury, inflammatory bowel disease, Alzheimer's disease and other neurodegenerative diseases. (All claimed).

ADMINISTRATION - The RAGE polypeptide is administered by oral route. No dosage given.

EXAMPLE - Cloning of receptor for advanced glycation endproducts isoform (RAGE) isoforms was carried out as follows. mRNA was isolated from human tissue types from healthy or diseased tissues or cell lines. mRNA was denatured at 70degreesC in the presence of 40% dimethylsulfoxide (DMSO) for 10 minutes and quenched on ice. First-strand cDNA was synthesized. After incubation at 37degreesC for 1 hour, the cDNA from both reactions were pooled and treated with 10 units of RNase H and PCR amplification was performed. PCR products were electrophoresed on a 1% agarose gel, and DNA from detectable bands was stained. The DNA bands were extracted with the QiaQuick gel extraction kit, ligated into the pDrive UA-cloning vector, and transformed into Escherichia coli. Recombinant plasmids were selected on LB agar plates containing 100 micrograms/ml carbenicillin. For each transfection, 192 colonies were randomly selected and their cDNA insert sizes were determined by PCR with M13 forward and reverse vector primers. Representative clones from PCR products with distinguishable molecular masses as visualized by fluorescence imaging were then sequenced from both directions with vector primers. All clones were sequenced entirely using custom primers for directed sequencing completion across gapped regions. Computational analysis of alternative splicing was performed by alignment of each cDNA sequence to its respective genomic sequence using SIM4. only transcripts with canonical (e.g. GT-AG) donor-acceptor splicing sites were considered for analysis. Clones encoding putative RAGE isoforms were obtained.(658 pages)

L6 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-15869 BIOTECHDS
TITLE: Protein-based compositions comprising a compound having at

least one therapeutic domain, and one anchoring domain, each comprising a peptide or protein, useful for treating or preventing pathogen infection, e.g. influenza;
involving vector-mediated gene transfer and expression in host cell for use in recombinant vaccine preparation

AUTHOR: YU M; FANG F
PATENT ASSIGNEE: YU M; FANG F
PATENT INFO: WO 2004047735 10 Jun 2004
APPLICATION INFO: WO 2003-US37158 21 Nov 2003
PRIORITY INFO: US 2003-464217 19 Apr 2003; US 2002-428535 22 Nov 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-441066 [41]

AB DERWENT ABSTRACT:

NOVELTY - A protein-based composition for preventing or treating infection by a pathogen, comprises a compound having at least one therapeutic domain (an enzyme) comprising a peptide or protein, and at least one anchoring domain comprising a peptide or protein.

DETAILED DESCRIPTION - A protein-based composition for preventing or treating infection by a pathogen, comprises a compound having at least one therapeutic domain comprising a peptide or protein, and at least one anchoring domain comprising a peptide or protein. The therapeutic domain has at least one extracellular activity that can prevent the infection of a target cell by a pathogen, and the anchoring domain can bind at or near the surface of a eukaryotic cell.

BIOTECHNOLOGY - Preferred Composition: The anchoring domain can bind at or near the surface of an epithelial or endothelial cell, preferably at or near the surface of an epithelial cell, where the anchoring domain binds an epithelial cell surface molecule. The epithelial cell surface molecule is a glycosaminoglycan, and anchoring domain can bind heparin or heparin sulfate, where the anchoring domain is a peptide comprising a GAG-binding amino acid sequence of a naturally-occurring protein, or a sequence that is substantially homologous to the GAG-binding sequence of a naturally-occurring protein. The peptide comprises the GAG-binding amino acid sequence of a mammalian protein, preferably a human protein. The peptide comprises an amino acid sequence substantially homologous to an amino acid sequence comprising 24, 27, 34, 34, 12 or 21 amino acids (SEQ ID NO: 2-7, respectively) given in the specification. The peptide comprises the GAG-binding amino acid sequence of human platelet factor 4 (SEQ ID NO: 2), human interleukin 8 (SEQ ID NO: 3), human antithrombin III (SEQ ID NO: 4), human apoprotein E (SEQ ID NO: 5), human angio-associated migratory protein (SEQ ID NO: 6), or human amphiregulin (SEQ ID NO: 7). The pathogen is a virus, such as an influenza virus, specifically an influenza A or an influenza B virus. The therapeutic domain comprises a protease inhibitor that inhibits an enzyme involved in processing a viral protein, where the enzyme involved in processing a viral protein is a host enzyme. The protease inhibitor is a serine protease inhibitor, preferably aprotinin, leupeptin, soybean protease inhibitor, e-aminocaproic acid, or n-p-tosyl-L-lysine. The anchoring domain is N-terminal to the therapeutic domain. There may also be at least 2 anchoring domains, where one of the 2 anchoring domains is N-terminal to the therapeutic domain, and at least one of the 2 anchoring domains is C-terminal to the one therapeutic domain. The 2 anchoring domains and at least one or two therapeutic domains are connected by peptide linkers. The therapeutic domain is an enzyme or its active portion, preferably a sialidase substantially homologous to at least a portion of at least one viral, bacterial or eukaryotic sialidase. The sialidase is substantially homologous to at least a portion of at least one bacterial sialidase, which is substantially homologous to at least a portion of a bacterial

sialidase that can cleave a sialic acid alpha, 2-6 linkage and a sialic acid alpha 2-3 linkage. The sialidase comprises or is substantially homologous to at least a portion of the sequence of Vibrio cholerae sialidase, Clostridium perfringens sialidase, Actinomyces viscosus sialidase, or Micromonospora viridifaciens sialidase. The sialidase is substantially homologous to at least a portion of NEU1, NEU3, NEU2 (SEQ ID NO: 8), or NEU4 (SEQ ID NO: 9). The composition further comprises at least one peptide linker that links the anchoring domain to the therapeutic domain, where the peptide linker comprises 1-100 amino acids, and comprises at least one glycine residue. Preferably, the peptide linker comprises the sequence (GGGGS)n, where n = a whole number from 1-20, particularly from 1-12 The formulation comprising the composition is formulated as a spray or as an inhalant. In using a sialidase to prevent or impede infection by a pathogen, the sialidase is substantially homologous to at least a portion of at least one viral, bacterial or eukaryotic sialidase. The subject is a human subject, and the sialidase is substantially homologous to at least a portion of at least one human sialidase. The sialidase is substantially homologous to at least a portion of NEU2 (379 amino acids, SEQ ID NO: 8), or NEU4 (424 amino acids, SEQ ID NO: 9). The composition comprising the sialidase is applied using a nasal spray, preferably an inhaler.

ACTIVITY - Virucide. Test details are described but no biological data given.

MECHANISM OF ACTION - Vaccine.

USE - The composition is useful for treating or preventing pathogen infection, particularly influenza infection (claimed).

ADMINISTRATION - The composition is applied using a nasal spray, preferably an inhaler, 1-4 times a day (claimed). The composition may also be administered topically, parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperitoneally, and at a dose of 1 ng/kg-10 mg/kg, preferably 100 ng/100 microg/kg.

ADVANTAGE - The new protein-based composition overcomes disadvantages of current therapies for treating or preventing pathogen infection, e.g. difficult to provide in a timely manner, undesirable effects, and can lead to drug-resistant pathogen strains.

EXAMPLE - Experimental protocols are described but no results are given. (75 pages)

L6 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2001:228746 HCAPLUS
DOCUMENT NUMBER: 134:261836
TITLE: Cell based assay for signal transduction comprising chimeric ligand-inducible transcription factors and its therapeutic application
INVENTOR(S): Zhong, Zhong; Kelly, Glen L.; Mercolino, Thomas J.; Zivin, Robert; Siekierka, John J.
PATENT ASSIGNEE(S): Ortho-McNeil Pharmaceutical, Inc., USA
SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001021215	A1	20010329	WO 2000-US25314	20000915

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW

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            CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
    CA 2383591          A1    20010329    CA 2000-2383591         20000915
    EP 1218036          A1    20020703    EP 2000-965037          20000915
        R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
            IE, SI, LT, LV, FI, RO, MK, CY, AL
    JP 2003509078       T     20030311    JP 2001-524638          20000915
    TR 200201530        T2    20030321    TR 2002-1530            20000915
    AU 782115           B2    20050707    AU 2000-75825           20000915
    US 2005244859       A1    20051103    US 2005-81049           20050314
PRIORITY APPLN. INFO.:                      US 1999-155353P     P   19990922
                                            US 2000-663306      B1  20000915
                                            WO 2000-US25314     W   20000915
AB  The present invention provides a whole-cell biol. assay that measures
    changes of endogenous genes under control of an exogenously introduced
    transcription factor.  The exogenous transcription factors of the present
    invention may be designed such that each is activated by specific
    extracellular ligands.  Therefore cells contg. exogenous
    transcription factors of the present invention provide a generic means to
    which many extracellular ligands may be tested without undue
    adaptation to the assay.  The invention is exemplified by measuring
    estradiol induction of EPO protein gene under the control of specific
    promoters mediated by a chimeric zinc finger transcription factor
    ZFP-ERLBD contg. ligand binding domain and transcription activation domain
    from estrogen receptor 1.alpha. and DNA binding domain specific to EPO
    protein gene promoter.  Transcription factor compns. related to interferon
    (IFN) signaling involved with Jak-STAT receptor pathway, dopamine
    signaling involved with G protein-coupled receptor, and PDGF signaling
    involved with receptor tyrosine kinase pathway are also described.  The
    method can be used for drug screening or analyzing drug effects.
REFERENCE COUNT:          5     THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS
                                RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> s therapeutic same anchoring domain
L7            0 THERAPEUTIC SAME ANCHORING DOMAIN

=> s therapeutic and anchoring domain?
L8           17 THERAPEUTIC AND ANCHORING DOMAIN?

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9            16 DUP REM L8 (1 DUPLICATE REMOVED)

=> s l9 and target?
L10           7 L9 AND TARGET?

=> d l10 1-7 ibib ab

L10  ANSWER 1 OF 7     MEDLINE on STN
ACCESSION NUMBER:   2006246973     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 16636283
TITLE:              Stably tethered multifunctional structures of defined
                    composition made by the dock and lock method for use in
                    cancer targeting.
AUTHOR:             Rossi Edmund A; Goldenberg David M; Cardillo Thomas M;
                    McBride William J; Sharkey Robert M; Chang Chien-Hsing
CORPORATE SOURCE:   IBC Pharmaceuticals, Inc., 300 American Road, Morris
                    Plains, NJ 07950, USA.
SOURCE:             Proceedings of the National Academy of Sciences of the
                    United States of America, (2006 May 2) Vol. 103, No. 18,
                    pp. 6841-6.  Electronic Publication: 2006-04-24.
                    Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY:       United States
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DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200607
ENTRY DATE: Entered STN: 4 May 2006
Last Updated on STN: 6 Jul 2006
Entered Medline: 5 Jul 2006

AB We describe a platform technology, termed the dock and lock method, which uses a natural binding between the regulatory subunits of cAMP-dependent protein kinase and the anchoring domains of A kinase anchor proteins for general application in constructing bioactive conjugates of different protein and nonprotein molecules from modular subunits on demand. This approach could allow quantitative and site-specific coupling of many different biological substances for diverse medical applications. The dock and lock method is validated herein by producing bispecific, trivalent-binding complexes composed of three stably linked Fab fragments capable of selective delivery of radiotracers to human cancer xenografts, resulting in rapid, significantly improved cancer targeting and imaging, providing tumor/blood ratios from 66 +/- 5 at 1 h to 395 +/- 26 at 24 h.

L10 ANSWER 2 OF 7 MEDLINE on STN
ACCESSION NUMBER: 2003479290 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14557453
TITLE: Unique epitopes of glutamic acid decarboxylase autoantibodies in slowly progressive type 1 diabetes.
AUTHOR: Kobayashi Tetsuro; Tanaka Shoichiro; Okubo Minoru; Nakanishi Koji; Murase Toshio; Lernmark Ake
CORPORATE SOURCE: Third Department of Internal Medicine, Yamanashi Medical University, Tamaho, Yamanashi 409-3898, Japan.. tetsurou@yamanashi.ac.jp
SOURCE: The Journal of clinical endocrinology and metabolism, (2003 Oct) Vol. 88, No. 10, pp. 4768-75.
Journal code: 0375362. ISSN: 0021-972X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200311
ENTRY DATE: Entered STN: 15 Oct 2003
Last Updated on STN: 13 Nov 2003
Entered Medline: 12 Nov 2003

AB Disease-specific epitope profiles of glutamic acid decarboxylase (GAD)65 autoantibodies (GAD65Ab) were studied in slowly progressive type 1 (insulin-dependent) diabetes mellitus (SPIDDM) and acute onset type 1 (insulin-dependent) diabetes mellitus (AIDDM) using seven kinds of GAD65/67 chimeric molecules. Sera obtained from Japanese SPIDDM (n = 17) and AIDDM (n = 46) patients followed prospectively were analyzed by immunoprecipitation, ELISA, and Western blotting. GAD65Ab in all SPIDDM samples reacted specifically with an N-terminal linear epitope located on the membrane anchoring domain between amino acids 17-51 and C-terminal conformational epitope between amino acids 443-585 of GAD65. The binding of GAD65Ab with N-terminal 83 residues in SPIDDM inversely correlated with the period in which insulin was not required. GAD65Ab in AIDDM did not react with N-terminal epitope located between amino acids 1-83, irrespective of the titer of GAD65Ab. A novel epitope of GAD65Ab in AIDDM residing between amino acids 244-360 was identified in 17% (8 of 46) of patients whose age of onset was younger than other AIDDM patients. In conclusion, GADAb in SPIDDM has unique N-terminal linear epitopes that are located on the anchoring domain of GAD65 molecules. Association is suggested between GAD65Ab targeted to this region and slowly progressive beta-cell failure in SPIDDM.

L10 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2005:450844 HCAPLUS
DOCUMENT NUMBER: 143:1221
TITLE: Antiviral proteins blocking infection using glycosaminoglycan-binding domains to bind protease inhibitors or sialidases to cell surfaces for treatment and preventing influenza
INVENTOR(S): Fang, Fang; Malakhov, Michael
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 82 pp., Cont.-in-part of U.S. Ser. No. 718,986.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005112751	A1	20050526	US 2004-939262	20040910
US 2005004020	A1	20050106	US 2003-718986	20031121
WO 2006031291	A2	20060323	WO 2005-US25831	20050721

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

PRIORITY APPLN. INFO.:

US 2002-428535P	P	20021122
US 2003-464217P	P	20030419
US 2003-718986	A2	20031121
US 2004-561749P	P	20040413
US 2004-580084P	P	20040616
US 2004-939262	A	20040910

AB Fusion proteins that use a glycosaminoglycan-binding domain to bind antibacterial proteins to a cell surface are described for the treatment of microbial infection, esp. influenza. Use of the glycosaminoglycan-binding domains targets the protein to the surface of epithelial cells, and this binds the therapeutic domain to the cell surface to prevent infection of the target cell by a pathogen such as an influenza virus. The glycosaminoglycan-binding anchoring domain may be from a mammalian protein, such as human platelet factor 4, interleukin 8, antithrombin III, or apolipoprotein E. The therapeutic domain may be an enzyme, such as a sialidase, or a protease inhibitor for a host enzyme involved in processing a viral protein. Examples of protease inhibitors are aprotinin, leupeptin, soybean proteinase inhibitor, e-aminocaproic acid, or n-p-tosyl-L-lysine.

L10 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2004:470946 HCAPLUS
DOCUMENT NUMBER: 141:33763
TITLE: Broad spectrum antivirals comprising a target cell-anchoring GAG-binding domain fused with protease inhibitor or sialidase, for treatment and preventing influenza
INVENTOR(S): Yu, Mang; Fang, Fang
PATENT ASSIGNEE(S): USA
SOURCE: PCT Int. Appl., 75 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004047735	A2	20040610	WO 2003-US37158	20031121
WO 2004047735	A3	20040923		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2506526	A1	20040610	CA 2003-2506526	20031121
AU 2003294401	A1	20040618	AU 2003-294401	20031121
EP 1567185	A2	20050831	EP 2003-789884	20031121
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
CN 1729013	A	20060201	CN 2003-80107241	20031121
JP 2006508193	T	20060309	JP 2005-510377	20031121

PRIORITY APPLN. INFO.:

US 2002-428535P	P	20021122
US 2003-464217P	P	20030419
WO 2003-US37158	W	20031121

AB The present invention provides new protein-based compns. and methods for preventing and treating pathogen infection, particularly influenza. The compds. have at least one N-terminal or C-terminal anchoring domain that anchors the compd. to the surface of a target epithelial cell, and at least one therapeutic domain that can act extracellularly to prevent infection of the target cell by a pathogen, such as a influenza virus. The said anchoring domain comprises a GAG-binding motif from a mammalian protein, such as human platelet factor 4, interleukin 8, antithrombin III, apolipoprotein E, angio-assocd. cell migratory protein (AAMP), or amphiregulin. The said therapeutic domain comprises enzyme, such as sialidase, or protease inhibitor for host enzyme involved in processing a viral protein. Examples of protease inhibitors are aprotinin, leupeptin, soybean proteinase inhibitor, e-aminocaproic acid, or n-p-tosyl-L-lysine.

L10 ANSWER 5 OF 7 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-06595 BIOTECHDS
TITLE: Producing antibody specifically binding protein domain in its native conformation by contacting minicell with cell competent for producing antibodies to antigen contacted with cell to generate immunogenic response;
vector-mediated single chain antibody gene transfer and expression in host cell for recombinant protein production
AUTHOR: SABBADINI R A; BERKLEY N; SURBER M W
PATENT ASSIGNEE: SABBADINI R A; BERKLEY N; SURBER M W
PATENT INFO: US 2003224444 4 Dec 2003
APPLICATION INFO: US 2002-157491 28 May 2002
PRIORITY INFO: US 2002-157491 28 May 2002; US 2002-359843 25 Feb 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-033964 [03]
AB DERWENT ABSTRACT:
NOVELTY - Producing (M1) an antibody (I) specifically binding a protein domain which is in its native conformation and contained within a protein displayed on a minicell comprising contacting the minicell with a cell, where the cell is competent for producing (I) to an antigen contacted

with the cell, in order to generate an immunogenic response in which the cell produces (I), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) producing (M2) derivative of (I) which specifically binds a protein domain, comprising contacting the minicell with a protein library, and identifying derivative of (I) from the protein library that specifically binds the protein domain; and (2) producing (M3) antibody or its derivative that specifically binds an epitope, comprising contacting a minicell displaying the epitope with a protein library or to a cell, where the cell is competent for producing (I) to an antigen contacted with the cell, in order to generate an immunogenic response in which the cell produces (I), and the epitope is chosen from an epitope composed of amino acids found within a membrane protein, an epitope present in an interface between a membrane protein and a membrane component, an epitope present in an interface between a membrane protein and one or more other proteins, and an epitope in a fusion protein comprising a first polypeptide which comprises a transmembrane domain, a membrane anchoring domain, and a second polypeptide comprising the epitope.

WIDER DISCLOSURE - (1) compositions and methods for preparing a soluble and/or secreted protein which remains in the cytoplasm of minicell; (2) a membrane protein not found naturally in a prokaryote; (3) biosensors comprising minicells; (4) preparing minicells; (5) minicell comprising an expression construct; (6) minicell comprising a biologically active compound, therapeutic agent; (7) pharmaceutical composition comprising minicell; (8) preparing a pharmaceutical composition; (9) device comprising microchips operatively associated with biosensor comprising a set of minicells; (10) minicell library comprising two or more minicells; (11) bio-remediation; (12) using minicells and its expression systems for manufacturing proteins on a large scale; (13) minicells having membrane protein having an intracellular domain; (14) minicells having membrane proteins linked to a conjugatable compound; (15) minicells used as research tools and/or kits comprising research tools; (16) L-form minicell; (17) producing a protein using minicells; (18) protoplast, poroplast comprising a vesicle; (19) a solid support comprising a minicell; (20) associating a radioactive compound with a cell; (21) delivering a biologically active compound to a cell; (22) minicell displaying a synthetic linking moiety; (23) sterically stabilized minicells; (24) minicell having a membrane comprising an exogenous lipid; (25) transferring a membrane protein from a minicell membrane to a biological membrane; (26) detecting an agent that is specifically bound by a binding moiety using minicell; (27) in situ imaging of a tissue or organ using minicell; (28) determining the rate of transfer of nucleic acid from a minicell to a cell; (29) determining the amount of nucleic acid transferred to a cell from a minicell; (30) detecting expression of an expression element in a cell using the minicell; (31) minicell comprising a nucleic acid; (32) introducing a nucleic acid into the cell using the minicell; (33) preparing minicell membrane; (34) determining three- dimensional structure of a membrane protein; (35) identifying ligand-interacting atoms in three-dimensional structure of a target protein; (36) identifying a nucleic acid encoding protein by using minicell library; (37) identifying undesirable side-effects of a biologically active compound that occur as a result of binding of compound to protein; (38) minicell-producing parent cell; (39) identifying an agent that affects the interaction of a first signaling protein with a second signaling protein; (40) immunogenic minicells that display an immunogen derived from non-functional, dysfunctional and/or diseased cell; (41) minicell comprising one or more bioactive nucleic acids or their templates; (42) minicells useful for in vivo drug delivery; (43) determining amino acid sequence of a protein that binds or chemically alters a preselected segment; and (44) episomal expression construct.

BIOTECHNOLOGY - Preferred Method: In (M1), the minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast. The protein displayed on minicell is a membrane protein which is a receptor

or a channel protein. The domain is found within the second polypeptide. The contacting step occurs in vivo, in an animal that comprises an adjuvant. In (M2), the protein library is chosen from phage display library, a phagemid display library and a ribosomal display library. The derivative of (I) is a single-chain antibody. In (M3), the protein library is contacted in vitro.

USE - (M1) is useful for producing antibody that specifically binds a protein domain which in its native conformation (claimed).

EXAMPLE - No suitable example given. (240 pages)

L10 ANSWER 6 OF 7 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04061 BIOTECHDS
TITLE: New minicells comprising a biologically active compound which displays a ligand or binding moiety, useful as vaccines or for treating e.g. cystic fibrosis, ulcerative colitis, rheumatoid arthritis, multiple sclerosis or cancer; poroplast and sheroplast minicell and antibody for use in disease therapy and gene therapy
AUTHOR: SABBADINI R A; KLEPPER R; SURBER M W
PATENT ASSIGNEE: SABBADINI R A; KLEPPER R; SURBER M W
PATENT INFO: US 2003211599 13 Nov 2003
APPLICATION INFO: US 2002-157106 28 May 2002
PRIORITY INFO: US 2002-157106 28 May 2002; US 2001-293566 24 May 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-901592 [82]
AB DERWENT ABSTRACT:

NOVELTY - A minicell comprising a biologically active compound which displays a ligand or binding moiety, where the ligand or binding moiety is part of a fusion protein comprising a first polypeptide that comprises at least one transmembrane domain or at least one membrane anchoring domain and a second polypeptide that comprises a binding moiety, where the minicell is a poroplast, sheroplast or protoplast, is new.

WIDER DISCLOSURE - Also disclosed are: (1) methods of using minicells and expression systems to manufacture on a large scale, proteins using recombinant DNA technology; (2) compositions and methods for preparing a soluble and/or secreted protein; (3) compositions and methods for preparing antibody or antibody derivative that recognize an immunogenic epitope present on a native form of a membrane protein, but which is not immunogenic when the membrane protein is denatured or when prepared as a synthetic oligopeptide; (4) biosensors comprising minicells; (5) methods of making minicells; (6) a method of producing a protein; (7) solid support comprising a minicell; (8) a method of associating a radioactive compound with a cell; (9) a method of delivering a biologically active compound to a cell; (10) a method of transferring a membrane protein from a minicell membrane to a biological membrane; (11) a pharmaceutical composition comprising a minicell; (12) a method of making a pharmaceutical composition or formulation; (13) a method of detecting an agent or substance that is specifically bound by a binding moiety; (14) a method of in situ imaging of a tissue or organ; (15) a method of identifying an agent that specifically binds a target compound; (16) a device comprising microchips operatively associated with a biosensor comprising a set of minicells in a prearranged pattern; (17) a method of determining the rate of transfer of a nucleic acid from a minicell to a cell; (18) a method of determining the amount of a nucleic acid transferred to a cell from a minicell; (19) a method of detecting the expression of an expression element in a cell; (20) methods of detecting the transfer of a fusion protein form a cytosol to an organelle of a eukaryotic cell; (21) a method of introducing a nucleic acid into a cell; 3a method of introducing into and expressing a nucleic acid in an organism; (22) a method of determining the three-dimensional structure of a membrane protein; (23) a method for identifying ligand-interacting atoms in a defined three-dimensional structure of a target protein; (24) a method of identifying a

nucleic acid that encodes a protein that binds to or chemically alters a pre-selected ligand; (25) a method of determining the amino acid sequence of a protein that binds or chemically alters a pre-selected ligand; (26) a method of identifying a nucleic acid that encodes a protein that inhibits or blocks an agent from binding to or chemically altering a pre-selected ligand; (27) a method of identifying an agent that effects the activity of a protein; (28) a method of identifying an agent that effects the activity of a protein domain containing a library of two or more candidate agents with a minicell displaying a membrane fusion protein; (29) a method for identifying an agent that effects the interaction of a first signaling protein with a second signaling protein; (30) a method of bioremediation; and (31) a minicell-producing parent cell.

BIOTECHNOLOGY - Preferred Minicell: The eubacterial minicell comprises a biologically active compound which displays a binding moiety, where the binding moiety is selected from a eukaryotic membrane protein, an archeabacterial membrane protein, an organellar membrane protein or a fusion protein comprising a first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain and a second polypeptide not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide comprising a binding moiety. The minicell further comprises a first and a second nucleic acid. The first nucleic acid comprises eukaryotic expression sequences operably linked to a first open reading frame (ORF) and the second nucleic acid comprises a eubacterial expression sequences operably linked to a second ORF. One of the ORF encodes a protein that comprises the binding moiety. The eubacterial expression sequences are induced and/or derepressed when the binding moiety is in contact with a target cell or when the nucleic acid is in the cytoplasm of a eukaryotic cell. The protein encoded by the first ORF comprises eukaryotic secretion sequences and/or the protein encoded by the second ORF comprises eubacterial secretion sequences. Preferred Compound: The biologically active compound is selected from a radioisotope, a polypeptide, a nucleic acid or a small molecule. Preferred Binding Moiety: The binding moiety is an antibody, an antibody derivative, a receptor or an active site of a non-catalytic derivative of an enzyme. The binding moiety is preferably a single chain antibody. It is directed to a ligand selected from an epitope displayed on a pathogen, an epitope displayed on an infected cell or an epitope displayed on a hyperproliferative cell. The ligand is directed to a receptor selected from epitope displayed on a pathogen, an epitope displayed on an infected cell or an epitope displayed on a hyperproliferative cell.

ACTIVITY - CNS-Gen.; Antiasthmatic; Respiratory-Gen.; Antiinflammatory; Antiulcer; Gastrointestinal-Gen.; Antiarthritic; Antirheumatic; Osteopathic; Neuroprotective; Immunosuppressive; Antidiabetic; Antidepressant; Neuroleptic; Nootropic; Antithyroid; Antiparkinsonian; Eating-Disorders-Gen.; Antibacterial; Virucide; Anti-HIV; Cytostatic; Protozoacide; Fungicide; Antiparasitic. No biological data given.

MECHANISM OF ACTION - Vaccine; Gene Therapy.

USE - Minicells are useful as vaccines to prevent or treat disease caused by intracellular pathogens or for treating diseases or disorders of the respiratory system including cystic fibrosis, asthma, hypersensitivity pneumonia, emphysema, bronchitis, sarcoid or interstitial lung disease; diseases or disorders of the digestive system including ulcerative colitis, gastroenteritis, inflammatory bowel disease or Crohn's disease; disorders or diseases of the skeletal system including spinal muscular atrophy, rheumatoid arthritis, osteoarthritis, osteoporosis or multiple myeloma-related bone disorder; autoimmune diseases including multiple sclerosis, Sjogren's syndrome, insulin dependent diabetes mellitus, autoimmune thyroiditis, amyotrophic lateral sclerosis, systemic lupus erythematosus or graft versus host disease; neurological diseases or disorders including depression, schizophrenia, Alzheimer's disease, Parkinson's disease, familial tremors, chronic pain or eating disorders; pathological diseases and resultant disorders

including bacterial infections, sepsis, septic shock, viral infections, HIV- or AIDS-related encephalitis or proliferative diseases including cancers. They can also be used to kill pathogenic protozoan, yeast and other fungi or parasitic worms. Minicells can also be used as diagnostic tools, in assays for screening pharmacological agents and in research applications.

ADMINISTRATION - Administration can be subcutaneous, intramuscular, intravenous or intradermal. No dosage given.

EXAMPLE - Escherichia coli were inoculated into bacterial growth media and grown overnight. The minicell producing cultures used to express protein post isolation were diluted and grown to the to the desired OD600 or OD450, typically in the log growth phase of bacterial cultures. The cultures were then induced with IPTG and then isolated. The IPTG concentration and exposure depended on which construct was being used, but was usually about 500 muM for a 4 hours. This treatment resulted in the production of the T7 polymerase, which was under control of the LacUVR5 promoter, which was repressed by the LacI repressor protein. IPTG relieves the LacI repression and induces expression from the LacUVR5 promoter which controls expression of the T7 polymerase from the chromosome. The E. coli cultures that produce minicells containing a therapeutic protein or nucleic acid have different protocols. The overnight cultures were diluted as described above, however, in the case of proteins that were not toxic to parent cells, this time the media used for dilution already contains IPTG. The cultures were then grown mid-log growth and minicells were isolated. These cultures produce the therapeutic protein or nucleic acid as they grow, and the minicells derived contained the therapeutic protein or nucleic acid. (243 pages)

L10 ANSWER 7 OF 7 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-01810 BIOTECHDS
TITLE: New minicell useful for producing achromosomal and anucleate cells for diagnosing or treating e.g. cancer, asthma, allergies, inflammation, diabetes, Alzheimer's disease or HIV, and as research tools and agents for drug discovery; minicell display and fusion protein for use in disease therapy and gene therapy
AUTHOR: SABBADINI R A; BERKLEY N; SURBER M W
PATENT ASSIGNEE: SABBADINI R A; BERKLEY N; SURBER M W
PATENT INFO: US 2003194714 16 Oct 2003
APPLICATION INFO: US 2002-157299 28 May 2002
PRIORITY INFO: US 2002-157299 28 May 2002; US 2001-295566 5 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-844449 [78]
AB DERWENT ABSTRACT:

NOVELTY - A minicell comprising at least one nucleic acid, is new.

DETAILED DESCRIPTION - The minicell displays a binding moiety directed to a target compound, where the binding moiety is selected from a eukaryotic membrane protein, an archeabacterial membrane protein, an organellar membrane protein, and a fusion protein. The fusion protein comprises a first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide that is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide, where the polypeptide comprises a binding moiety. AN INDEPENDENT CLAIM is included for the method of introducing a nucleic acid into a cell, comprising contacting the cell with the minicell cited above.

WIDER DISCLOSURE - (1) compositions and methods for preparing the minicells, a soluble and/or secreted protein, or antibodies and/or antibody derivatives that recognize an immunogenic epitope on the native form of a membrane protein; (2) a method of associating a radioactive compound with a cell; (3) a method of transferring a membrane protein from a minicell membrane to a biological membrane; (4) a pharmaceutical

composition comprising the minicell; (5) a method of making the above pharmaceutical composition; (6) a method of detecting an agent that is specifically bound by a binding moiety; (7) a method of in situ imaging of a tissue or organ; (8) methods of determining the rate or amount of transfer of nucleic acid from a minicell to a cell; (9) a method of determining the three-dimensional structure of a membrane protein; (10) a method of identifying ligand-interacting atoms in a defined three-dimensional structure of a target protein; (11) methods of identifying a nucleic acid that encodes the above protein; and (12) methods of bioremediation.

BIOTECHNOLOGY - Preferred Minicell: The minicell is selected from a eubacterial minicell, a poroplast, a spheroplast and a protoplast. The nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF encoding the proteins mentioned above or encoding a therapeutic polypeptide. The therapeutic polypeptide is a membrane polypeptide or a soluble polypeptide. The soluble polypeptide comprises a cellular secretion sequence. The expression sequences are inducible and/or repressible. These are induced and/or depressed when the binding moiety displayed by the minicell binds to its target compound. The ORF encodes a polypeptide having an amino acid sequence that facilitates cellular transfer of a biologically active compound contained within or displayed by the minicell. The membrane of the minicell comprises a system for transferring a molecule from the interior of a minicell into the cytoplasm of the cell. The system is a Type III secretion system. Preferred Method: In the above method, the expression sequences are induced or derepressed by a transactivation or transrepression event.

ACTIVITY - Cytostatic; Antiasthmatic; Antiallergic; Antiinflammatory; Antirheumatic; Antiarthritic; Antidiabetic; Neuroprotective; Nootropic; Antiparkinsonian; Anti-HIV; Antibacterial; Hepatotropic; Vasotropic; Cardiant. No biological data given.

MECHANISM OF ACTION - Cell therapy.

USE - The minicell and method are useful in producing achromosomal and anucleate cells for diagnostic and therapeutic applications (e.g. in diagnosing or treating cancer, asthma, allergies, inflammation, rheumatoid arthritis, diabetes, Alzheimer's disease, Parkinson's disease, HIV, bacterial infections, hepatitis or myocardial ischemia), as well as research tools and agents for drug discovery or for delivery of nucleic acids and other bioactive compounds to cells.

ADMINISTRATION - Administration can be oral, pulmonary, nasal, buccal, ocular, dermal, rectal or vaginal. No dosage given.

EXAMPLE - No relevant example given. (244 pages)

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=> s broad spectrum antiviral
L11         762 BROAD SPECTRUM ANTIVIRAL

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12          346 DUP REM L11 (416 DUPLICATES REMOVED)

=> s l12 and fusion protein?
L13            1 L12 AND FUSION PROTEIN?

=> d l13 ibib ab

L13  ANSWER 1 OF 1      MEDLINE on STN
ACCESSION NUMBER:    2005505379      MEDLINE
DOCUMENT NUMBER:     PubMed ID: 16155572
TITLE:               Carbohydrate-binding molecules inhibit viral fusion and
                     entry by crosslinking membrane glycoproteins.
AUTHOR:              Leikina Eugenia; Delanoe-Ayari Helene; Melikov Kamran; Cho
                     Myoung-Soon; Chen Andrew; Waring Alan J; Wang Wei; Xie
                     Yongming; Loo Joseph A; Lehrer Robert I; Chernomordik
                     Leonid V
```

CORPORATE SOURCE: Section on Membrane Biology, Laboratory of Cellular and Molecular Biophysics, National Institute of Child Health and Human Development, Bethesda, Maryland 20892-1855, USA.
CONTRACT NUMBER: AI056921 (NIAID)
RR 20004 (NCRR)
SOURCE: Nature immunology, (2005 Oct) Vol. 6, No. 10, pp. 995-1001. Electronic Publication: 2005-09-11. Journal code: 100941354. ISSN: 1529-2908.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200511
ENTRY DATE: Entered STN: 23 Sep 2005
Last Updated on STN: 3 Nov 2005
Entered Medline: 2 Nov 2005

AB Defensins are peptides that protect the host against microorganisms. Here we show that the theta-defensin retrocyclin 2 (RC2) inhibited influenza virus infection by blocking membrane fusion mediated by the viral hemagglutinin. RC2 was effective even after hemagglutinin attained a fusogenic conformation or had induced membrane hemifusion. RC2, a multivalent lectin, prevented hemagglutinin-mediated fusion by erecting a network of crosslinked and immobilized surface glycoproteins. RC2 also inhibited fusion mediated by Sindbis virus and baculovirus. Human beta-defensin 3 and mannan-binding lectin also blocked viral fusion by creating a protective barricade of immobilized surface proteins. This general mechanism might explain the broad-spectrum antiviral activity of many multivalent lectins of the innate immune system.

=> s l12 and polypeptide domain?
L14 0 L12 AND POLYPEPTIDE DOMAIN?

=> s l12 and binding domain?
L15 2 L12 AND BINDING DOMAIN?

=> d l15 1-2

L15 ANSWER 1 OF 2 MEDLINE on STN
AN 2001330748 MEDLINE
DN PubMed ID: 11384233
TI 2-Amino-6-arylsulfonylbenzonitriles as non-nucleoside reverse transcriptase inhibitors of HIV-1.
AU Chan J H; Hong J S; Hunter R N 3rd; Orr G F; Cowan J R; Sherman D B; Sparks S M; Reitter B E; Andrews C W 3rd; Hazen R J; St Clair M; Boone L R; Ferris R G; Creech K L; Roberts G B; Short S A; Weaver K; Ott R J; Ren J; Hopkins A; Stuart D I; Stammers D K
CS Glaxo Wellcome, Inc., 5 Moore Drive, Research Triangle Park, North Carolina 27709, USA.. jc25572@glaxowellcome.com
SO Journal of medicinal chemistry, (2001 Jun 7) Vol. 44, No. 12, pp. 1866-82. Journal code: 9716531. ISSN: 0022-2623.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200106
ED Entered STN: 2 Jul 2001
Last Updated on STN: 2 Jul 2001
Entered Medline: 28 Jun 2001

L15 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 2001:350623 BIOSIS
DN PREV200100350623
TI Mutations in the adenovirus type 5 viral DNA polymerase that lead to

resistance to the broad spectrum antiviral
Cidofovir.
AU Kinchington, P. R. [Reprint author]; Araullo-Cruz, T. [Reprint author]; Vergnes, J. P. [Reprint author]; Gordon, Y. J. [Reprint author]
CS Charles T. Campbell Laboratory, Ophthalmology, Univ Pittsburgh, Pittsburgh, PA, USA
SO IOVS, (March 15, 2001) Vol. 42, No. 4, pp. S578. print.
Meeting Info.: Annual Meeting of the Association for Research in Vision and Ophthalmology. Fort Lauderdale, Florida, USA. April 29-May 04, 2001. Association for Research in Vision and Ophthalmology.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 25 Jul 2001
Last Updated on STN: 19 Feb 2002

=> s antiviral compound? and anchoring and therapeutic
L16 0 ANTIVIRAL COMPOUND? AND ANCHORING AND THERAPEUTIC

=> s antiviral compound? and anchoring
L17 0 ANTIVIRAL COMPOUND? AND ANCHORING

=> s antiviral and anchoring and therapeutic
L18 9 ANTIVIRAL AND ANCHORING AND THERAPEUTIC

=> s antiviral and anchoring and therapeutic and binding
L19 4 ANTIVIRAL AND ANCHORING AND THERAPEUTIC AND BINDING

=> d l18 1-9 ibib ab

L18 ANSWER 1 OF 9 MEDLINE on STN
ACCESSION NUMBER: 2006596455 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16979654
TITLE: Suppression of HIV-1 protease inhibitor resistance by phosphonate-mediated solvent anchoring.
AUTHOR: Cihlar Tomas; He Gong-Xin; Liu Xiaohong; Chen James M; Hatada Marcos; Swaminathan Swami; McDermott Martin J; Yang Zheng-Yu; Mulato Andrew S; Chen Xiaowu; Leavitt Stephanie A; Stray Kirsten M; Lee William A
CORPORATE SOURCE: Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, USA.
SOURCE: Journal of molecular biology, (2006 Oct 27) Vol. 363, No. 3, pp. 635-47. Electronic Publication: 2006-08-02.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-2I4D; PDB-2I4U; PDB-2I4V; PDB-2I4W; PDB-2I4X
ENTRY MONTH: 200612
ENTRY DATE: Entered STN: 11 Oct 2006
Last Updated on STN: 19 Dec 2006
Entered Medline: 18 Dec 2006

AB The introduction of human immunodeficiency virus type 1 (HIV-1) protease inhibitors (PIs) markedly improved the clinical outcome and control of HIV-1 infection. However, cross-resistance among PIs due to a wide spectrum of mutations in viral protease is a major factor limiting their broader clinical use. Here we report on the suppression of PI resistance using a covalent attachment of a phosphonic acid motif to a peptidomimetic inhibitor scaffold. The resulting phosphonate analogs maintain high binding affinity to HIV-1 protease, potent antiretroviral activity, and unlike the parent molecules, display no loss of potency against a panel of clinically important PI-resistant HIV-1 strains. As shown by crystallographic analysis, the phosphonate moiety is highly exposed to

solvent with no discernable interactions with any of the enzyme active site or surface residues. We term this effect "solvent anchoring " and demonstrate that it is driven by a favorable change in the inhibitor binding entropy upon the interaction with mutant enzymes. This type of thermodynamic behavior, which was not found with the parent scaffold fully buried in the enzyme active site, is a result of the increased degeneracy of inhibitor binding states, allowing effective molecular adaptation to the expanded cavity volume of mutant proteases. This strategy, which is applicable to various PI scaffolds, should facilitate the design of novel PIs and potentially other antiviral therapeutics.

L18 ANSWER 2 OF 9 MEDLINE on STN
ACCESSION NUMBER: 96182508 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8600417
TITLE: Ganciclovir intraocular implant. A clinicopathologic study.
AUTHOR: Charles N C; Steiner G C
CORPORATE SOURCE: Department of Ophthalmology, New York University Medical Center, USA.
SOURCE: Ophthalmology, (1996 Mar) Vol. 103, No. 3, pp. 416-21. Journal code: 7802443. ISSN: 0161-6420.
PUB. COUNTRY: United States
DOCUMENT TYPE: (CASE REPORTS)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199605
ENTRY DATE: Entered STN: 13 May 1996
Last Updated on STN: 6 Feb 1998
Entered Medline: 2 May 1996

AB BACKGROUND: Surgical implantation of the intraocular sustained-release ganciclovir device is a safe and effective treatment for cytomegalovirus (CMV) retinitis. Previous histopathologic studies on eyes containing such implants have been limited by the necessity of removing the device before processing. Microtome sectioning of hard plastics within paraffin-embedded blocks is infeasible, and the anatomic relations of implant to eye are destroyed. METHODS: The authors studied four eyes from three patients who had undergone implant insertion. Globes with implants in place were fixed in neutral 10% formation, embedded in methylmethacrylate, sectioned on a special microtome, and stained with hematoxylin-eosin. RESULTS: After methacrylate embedding, the precise anatomic relations of the implant to the neighboring uveoscleral coats were preserved. In two eyes, the suture tab of the implant protruded through the sclera, exiting subconjunctivally. In two eyes, the implant was totally intravitreal. In all patients, the device was supported by fibrous tissue which emanated from a surgical coloboma of the pars plana ciliaris. Focal granulomatous inflammation adjoined suture and implant materials but no other inflammation or deleterious effects on the ocular structures were noted. CONCLUSION: This report is the first to document the intraocular histopathology of the ganciclovir implant. The subconjunctival location, enhancing the potential for endophthalmitis, may be avoided by trimming of the suture tab close to the anchoring suture and not tying it too tightly. Methylmethacrylate embedding is a useful technique for preserving the microanatomy of intraocular implants.

L18 ANSWER 3 OF 9 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2006:1063109 HCAPLUS
DOCUMENT NUMBER: 145:413661
TITLE: Stably tethered structures of defined composition with multiple functions or binding specificities for disease diagnosis and treatment
INVENTOR(S): Chien, Hsing Chang; Goldenberg, David M.; McBride, William J.; Rossi, Edmund A.
PATENT ASSIGNEE(S): Ibc Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 166pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2006107786	A2	20061012	WO 2006-US12084		20060329
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW					
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
US 2006228300	A1	20061012	US 2006-391584		20060328
PRIORITY APPLN. INFO.:			US 2005-668603P	P	20050406
			US 2005-728292P	P	20051019
			US 2005-751196P	P	20051216
			US 2006-782332P	P	20060314
			US 2006-391584	A	20060328

AB The present invention concerns methods and compns. for stably tethered structures of defined compns. with multiple functionalities and/or binding specificities. Particular embodiments concern stably tethered structures comprising a homodimer of a first monomer, comprising a dimerization and docking domain (DDD) attached to a first precursor, and a second monomer comprising an anchoring domain (AD) attached to a second precursor. The first and second precursors may be virtually any mol. or structure, such as antibodies, antibody fragments, antibody analogs or mimetics, aptamers, binding peptides, fragments of binding proteins, known ligands for proteins or other mols., enzymes, detectable labels or tags, therapeutic agents, toxins, pharmaceuticals, cytokines, interleukins, interferons, radioisotopes, proteins, peptides, peptide mimetics, polynucleotides, RNAi, oligosaccharides, natural or synthetic polymeric substances, nanoparticles, quantum dots, org. or inorg. compds., etc. The disclosed methods and compns. provide a simple, easy to purify way to obtain any binary compd. attached to any monomeric compd., or any trinary compd. Thus, an anti-CEA Fab fused to a DDD sequence from the regulatory subunit of cAMP-dependent protein kinase was prepd. with transgenic cells and shown to form dimers. The stability of these dimers can be increased by, for example, incorporating cysteine residues into the DDD peptide such that, when the dimer is formed, the cysteine residues are brought into proximity and can thereby form disulfide bonds. To demonstrate that these Fab dimers may be used to "pretarget" tumor cells, the dimers were injected into tumor-bearing mice and were shown to conc. at the site of the tumor. Injection of a peptide contg. an A kinase anchoring protein peptide (AD) which is fused to an imaging agent or therapeutic agent is expected to lead to localization of this conjugate at the tumor due to interaction of the AD and DDD domains.

L18 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2005:1242684 HCAPLUS
DOCUMENT NUMBER: 143:474231
TITLE: Soluble derivatives of human neutral hyaluronidase and preparation with transgenic cells for use in therapeutic modulation of glycosaminoglycan metabolism
INVENTOR(S): Bookbinder, Louis H.; Kundu, Anirban; Frost, Gregory I.; Haller, Michael F.; Keller, Gilbert A.; Dylan, Tyler M.

PATENT ASSIGNEE(S): Halozyme, Inc., USA
SOURCE: U.S. Pat. Appl. Publ., 121 pp., Cont.-in-part of U.S. Ser. No. 795,095.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2005260186	A1	20051124	US 2005-65716	20050223
US 2004268425	A1	20041230	US 2004-795095	20040305
US 2006104968	A1	20060518	US 2005-238171	20050927
WO 2006091871	A1	20060831	WO 2006-US6700	20060223

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

PRIORITY APPLN. INFO.:

US 2003-452360P	P	20030305
US 2004-795095	A2	20040305
US 2005-65716	A2	20050223
US 2005-238171	A	20050927

AB The invention relates to the discovery of novel sol. neutral active Hyaluronidase Glycoproteins (sHASEGPs), methods of manuf., and their use to facilitate administration of other mols. or to alleviate glycosaminoglycan assocd. pathologies. Minimally active polypeptide domains of the sol., neutral active sHASEGP domains are described that include asparagine-linked sugar moieties required for a functional neutral active hyaluronidase domain. Included are modified amino-terminal leader peptides that enhance secretion of sHASEGP. The invention further comprises sialated and pegylated forms of a recombinant sHASEGP to enhance stability and serum pharmacokinetics over naturally occurring slaughterhouse enzymes. Further described are suitable formulations of a substantially purified recombinant sHASEGP glycoprotein derived from a eukaryotic cell that generate the proper glycosylation required for its optimal activity.

L18 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2005:450844 HCAPLUS
DOCUMENT NUMBER: 143:1221
TITLE: Antiviral proteins blocking infection using glycosaminoglycan-binding domains to bind protease inhibitors or sialidases to cell surfaces for treatment and preventing influenza
INVENTOR(S): Fang, Fang; Malakhov, Michael
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 82 pp., Cont.-in-part of U.S. Ser. No. 718,986.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

US 2005112751	A1	20050526	US 2004-939262		20040910
US 2005004020	A1	20050106	US 2003-718986		20031121
WO 2006031291	A2	20060323	WO 2005-US25831		20050721

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

PRIORITY APPLN. INFO.:	US 2002-428535P	P	20021122
	US 2003-464217P	P	20030419
	US 2003-718986	A2	20031121
	US 2004-561749P	P	20040413
	US 2004-580084P	P	20040616
	US 2004-939262	A	20040910

AB Fusion proteins that use a glycosaminoglycan-binding domain to bind antibacterial proteins to a cell surface are described for the treatment of microbial infection, esp. influenza. Use of the glycosaminoglycan-binding domains targets the protein to the surface of epithelial cells, and this binds the therapeutic domain to the cell surface to prevent infection of the target cell by a pathogen such as an influenza virus. The glycosaminoglycan-binding anchoring domain may be from a mammalian protein, such as human platelet factor 4, interleukin 8, antithrombin III, or apolipoprotein E. The therapeutic domain may be an enzyme, such as a sialidase, or a protease inhibitor for a host enzyme involved in processing a viral protein. Examples of protease inhibitors are aprotinin, leupeptin, soybean proteinase inhibitor, e-aminocaproic acid, or n-p-tosyl-L-lysine.

L18 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER:	2004:754417 HCAPLUS
DOCUMENT NUMBER:	141:256532
TITLE:	Soluble derivatives of human neutral hyaluronidase and their secretory manufacture for use in therapeutic modulation of glycosaminoglycan metabolism
INVENTOR(S):	Bookbinder, Louis H.; Kundu, Anirban; Frost, Gregory I.
PATENT ASSIGNEE(S):	Deliatroph Pharmaceuticals, Inc., USA
SOURCE:	PCT Int. Appl., 210 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004078140	A2	20040916	WO 2004-US6656	20040305

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI

RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

CA 2517145	A1	20040916	CA 2004-2517145	20040305
EP 1603541	A2	20051214	EP 2004-717941	20040305

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK

BR 2004008116	A	20060301	BR 2004-8116		20040305
JP 2006524507	T	20061102	JP 2006-509139		20040305
PRIORITY APPLN. INFO.:			US 2003-452360P	P	20030305
			WO 2004-US6656	W	20040305

AB A variant of human neutral active hyaluronidase with improved soly. is constructed and a cDNA encoding it is cloned for manuf. of the enzyme for use in the the treatment of glycosaminoglycan-assocd. pathologies. This variant of the enzyme lacks its hydrophobic C-terminal domain including the GPI anchor to improve soly. and increase yields of secreted activity. Minimally active domains of the enzyme, including asparagine-linked glycosidation required for a functional enzyme are identified. Secretory manuf. of the enzyme and the use of leader peptides that increase the efficiency of secretion of the enzyme are also described. The signal and leader peptide of the enzyme is unusually long and may play a role in limiting secretion by promoting aggregation. Replacing it with the signal peptide of the mouse Ig .kappa. chain increased yields of secreted enzyme by .apprx.6-fold. Modified forms of the enzyme, e.g. sialylated and PEGylated, with increased stability and serum pharmacokinetics over naturally occurring slaughterhouse enzymes are described. Further described are suitable formulations of a substantially purified recombinant sHASEGP glycoprotein derived from a eukaryotic cell that generate the proper glycosylation required for its optimal activity.

L18 ANSWER 7 OF 9 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER:	2004:470946 HCAPLUS
DOCUMENT NUMBER:	141:33763
TITLE:	Broad spectrum antivirals comprising a target cell-anchoring GAG-binding domain fused with protease inhibitor or sialidase, for treatment and preventing influenza
INVENTOR(S):	Yu, Mang; Fang, Fang
PATENT ASSIGNEE(S):	USA
SOURCE:	PCT Int. Appl., 75 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	2
PATENT INFORMATION:	

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 2004047735	A2	20040610	WO 2003-US37158		20031121
WO 2004047735	A3	20040923			
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW					
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG					
CA 2506526	A1	20040610	CA 2003-2506526		20031121
AU 2003294401	A1	20040618	AU 2003-294401		20031121
EP 1567185	A2	20050831	EP 2003-789884		20031121
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK					
CN 1729013	A	20060201	CN 2003-80107241		20031121
JP 2006508193	T	20060309	JP 2005-510377		20031121
PRIORITY APPLN. INFO.:			US 2002-428535P	P	20021122
			US 2003-464217P	P	20030419
			WO 2003-US37158	W	20031121

AB The present invention provides new protein-based compns. and methods for preventing and treating pathogen infection, particularly influenza. The compds. have at least one N-terminal or C-terminal anchoring domain that anchors the compd. to the surface of a target epithelial cell, and at least one therapeutic domain that can act extracellularly to prevent infection of the target cell by a pathogen, such as a influenza virus. The said anchoring domain comprises a GAG-binding motif from a mammalian protein, such as human platelet factor 4, interleukin 8, antithrombin III, apolipoprotein E, angio-assocd. cell migratory protein (AAMP), or amphiregulin. The said therapeutic domain comprises enzyme, such as sialidase, or protease inhibitor for host enzyme involved in processing a viral protein. Examples of protease inhibitors are aprotinin, leupeptin, soybean proteinase inhibitor, e-aminocaproic acid, or n-p-tosyl-L-lysine.

L18 ANSWER 8 OF 9 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1993:603859 HCAPLUS
DOCUMENT NUMBER: 119:203859
TITLE: Preparation of lipid conjugates of therapeutic peptides and protease inhibitors
INVENTOR(S): Basava, Channa; Hostetler, Karl Y.
PATENT ASSIGNEE(S): Vical, Inc., USA
SOURCE: PCT Int. Appl., 72 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9301828	A1	19930204	WO 1992-US6153	19920722
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE				
US 5554728	A	19960910	US 1991-734434	19910723
CA 2113156	A1	19930204	CA 1992-2113156	19920722
AU 9224251	A	19930223	AU 1992-24251	19920722
AU 671078	B2	19960815		
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R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE				
JP 07501316	T	19950209	JP 1992-503064	19920722
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OTHER SOURCE(S): MARPAT 119:203859
AB Title compds., comprising therapeutic peptides, including human immunodeficiency virus (HIV) protease inhibitors covalently linked to phospholipids, glycerides, or other membrane-targeting and membrane-anchoring species, and their pharmaceutically acceptable salts, together with processes for their prepns., are described. The invention also provides novel HIV protease inhibitors. The prepd. compds. possess useful pharmacol. properties, such as antiviral activity towards viral infection and inhibitory activity towards viral proteases. Therefore, these compds. can be used in the prophylaxis or treatment of viral infections, in particular infections caused by HIV or other retroviruses. The targeting technol. as described for the protease inhibitors is also applicable to a variety of inhibitors of other enzymes. Thus, R-Ala-Ala-D-.beta.-Nal-Pip-OMe (I; R = Ac, .beta.-Nal = .beta.-naphthylalanine, Pip = pipecolic acid), prepd. by std. solid-phase methods, had IC50 >100 .mu.M in an antiviral assay, while dipalmitoylglycerophosphatidylethanolamine conjugate I [R = (R)-Me(CH2)14CO2CH[CH2O2C(CH2)14Me]CH2OP(O)(OH)OCH2CH2NHCOCH2CH2CO], prepd. via coupling of succinylated ethanolamine deriv. ROH with the corresponding peptide, had IC50 = 10 .mu.M.

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AUTHOR(S): Hu, Jian-Da [Reprint author]; Schmah, Oliver [Reprint author]; Finke, Juergen [Reprint author]; Veelken, Hendrik [Reprint author]
CORPORATE SOURCE: Department of Hematology/Oncology, Freiburg University Medical Center, Freiburg, Germany
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AB Donor lymphocyte infusions (DLI) are currently standard treatment for recurrent hematologic malignancy after allogeneic stem cell transplantation. A major limitation of DLI is the risk of inducing severe graft-versus-host disease (GvHD). Transduction of donor T cells with the "suicide gene" HSV thymidine kinase (TK) prior to DLI is an attractive approach to conditionally ablate GvHD-inducing donor T cells in vivo by systemic treatment with the prodrug gancyclovir (GCV). Genetic modification of primary T cells may be achieved with retroviral vectors, however, limited transduction efficiencies usually mandate a positive in vitro selection step to ensure transduction of all adoptively transferred T cells. Positive selection can be achieved by coexpression of TK with a drug resistance gene such as neomycin phosphotransferase. Alternatively, the cell surface molecules LNGFR and CD34 have been proposed for rapid immunoselection of transduced T cells. We have constructed a MMLV-based retrovirus vector (LXTK20) encoding HSV TK and the B cell surface molecule CD20 in a dicistronic expression cassette. Translation of the downstream CD20 cistron is achieved by fusion to the internal ribosome entry sequence of EMCV virus. This vector design virtually ensures TK activity in CD20-positive cells. The choice of CD20 as a selectable marker was based on (1) low expression levels and lack of a known function in human T cells, (2) availability of immunomagnetic sorting reagents, (3) firm anchoring and lack of shedding due to four transmembrane domains, and (4) availability of a anti-CD20 antibody licensed for in vivo therapy (rituximab) which may be combined with GCV to enhance the efficacy of T cell elimination. Infectious LXTK20 supernatants were obtained by calcium phosphate transfection of Phoenix packaging cells. After incubation of NIH3t3 cells with infectious LXTK20 particles, transduced cells could be selected efficiently with magnetic beads coupled to anti-CD20-mAb. Immunophenotyping revealed stable expression of CD20 over a four week expansion period. As assessed by a metabolic XTT assay, growth of LXTK20-transduced, CD20-positive cells was completely inhibited by treatment with 1 mg/L GCV for 4 days. These data indicate that the retroviral vector LXTK20 facilitates efficient and rapid sorting of transduced cells and confers high sensitivity to GCV. LXTK20 appears to be a promising agent for further preclinical and clinical developments of adoptive transfer of suicide gene-transduced T cells.

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CHAPTER

1 PHARMACOKINETICS: THE DYNAMICS OF DRUG ABSORPTION, DISTRIBUTION, AND ELIMINATION

Leslie Z. Benet, Jerry R. Mitchell, and Lewis B. Sheiner

To produce its characteristic effects, a drug must be present in appropriate concentrations at its sites of action. Although obviously a function of the amount of drug administered, the concentrations attained also depend upon the extent and rate of its absorption, distribution, binding or localization in tissues, biotransformation, and excretion. These factors are depicted in Figure 1–1.

PHYSICOCHEMICAL FACTORS IN TRANSFER OF DRUGS ACROSS MEMBRANES

The absorption, distribution, biotransformation, and excretion of a drug all involve its passage across cell membranes. It is essential, therefore, to consider the mechanisms by which drugs cross membranes and the physicochemical properties of molecules and membranes that influence this transfer. Important characteristics of a drug are its molecular size and shape, solubility at the site of its absorption, degree of ionization, and relative lipid solubility of its ionized and nonionized forms.

When a drug permeates a cell, it must obviously traverse the cellular plasma membrane. Other barriers to drug movement may be a single layer of cells (intestinal epithelium) or several layers of cells (skin). Despite these structural differences, the diffusion and transport of drugs across these various boundaries have many common characteristics, since drugs in general pass through cells rather than between

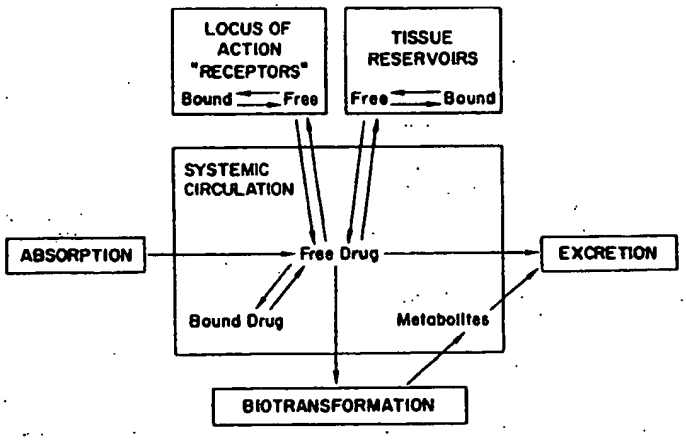


Figure 1–1. *Schematic representation of the interrelationship of the absorption, distribution, binding, biotransformation, and excretion of a drug and its concentration at its locus of action.*

Possible distribution and binding of metabolites are not depicted.

them. The plasma membrane thus represents the common barrier.

Cell Membranes. Initially, cell membranes were hypothesized to consist of a thin layer of lipid-like material interspersed with minute water-filled channels. Subsequent studies suggested that the plasma membrane consisted of a bilayer of amphipathic lipids, with their hydrocarbon chains oriented inward to form a continuous hydrophobic phase and their hydrophilic heads oriented outward. This hypothesis has been broadened to a more dynamic fluid-mosaic model, where globular protein molecules penetrate into either side of or entirely through a fluid phospholipid bilayer (Singer and Nicolson, 1972). Individual lipid molecules in the bilayer can move laterally, endowing the membrane with fluidity, flexibility, high electrical resistance, and relative impermeability to highly polar molecules. However, it is also appreciated that complexes of intrinsic membrane proteins and lipids can form either hydrophilic or hydrophobic channels that allow transport of molecules with different characteristics.

Passive Processes. Drugs cross membranes either by passive processes or by mechanisms involving the active participation of components of the membrane. In the former, the drug molecule usually penetrates by passive diffusion along a concentration gradient by virtue of its solubility in the lipid bilayer. Such transfer is directly proportional to the magnitude of the concentration gradient across the membrane and the lipid:water partition coefficient of the drug. The greater the partition coefficient, the higher is the concentration of drug in the membrane and the faster is its diffusion. After a steady state is attained, the concentration of the free drug is the same on both sides of the membrane, if the drug is a nonelectrolyte. For ionic compounds, the steady-state concentrations will be dependent on differences in pH across the membrane, which may influence the state of ionization of the molecule on each side of the membrane, and on the electrochemical gradient for the ion. Most biological membranes are relatively permeable to water, either by diffusion or by flow that results from hydrostatic or osmotic differences across the membrane. Such bulk flow of water can carry with it small, water-soluble substances. Most cell membranes permit passage only of water, urea, and other small, water-soluble molecules by this mechanism. Such substances generally do not pass through cell membranes if their molecular weights are greater than 100 to 200.

While most inorganic ions would seem to be sufficiently small to penetrate the membrane, their hydrated ionic radius is relatively large. The concentration gradient of many inorganic ions is largely determined by active transport (*e.g.*, Na^+ and K^+). The transmembrane potential frequently determines the distribution of other ions (*e.g.*, chloride) across the membrane. Channels with selectivity for individual ions are often controlled to allow regulation of specific ionic fluxes. Such mechanisms are of obvious importance in the generation of action potentials in nerve and muscle (*see* Chapter 5) and in transmembrane signaling events (*see* Chapter 2).

Weak Electrolytes and Influence of pH. Most drugs are weak acids or bases that are present in solution as both the nonionized and ionized species. The nonionized molecules are usually lipid soluble and can diffuse across the cell membrane. In contrast, the ionized molecules are usually unable to penetrate the lipid membrane because of their low lipid solubility.

Therefore, the transmembrane distribution of a weak electrolyte is usually determined by its pK_a and the pH gradient across the membrane. To illustrate the effect of pH on distribution of drugs, the partitioning of a weak acid ($pK_a = 4.4$) between plasma (pH = 7.4) and gastric juice (pH = 1.4) is depicted in Figure 1–2. It is assumed that the gastric mucosal membrane behaves as a simple lipid barrier that is permeable only to the lipid-soluble, nonionized form of the acid. The ratio of nonionized to ionized drug at each pH is easily calculated from the Henderson–Hasselbalch equation. Thus, in plasma, the ratio of nonionized to ionized drug is 1:1000; in gastric juice, the ratio is 1:0.001. These values are given in brackets in Figure 1–2. The total concentration ratio between the plasma and the gastric juice would therefore be 1000:1 if such a system came

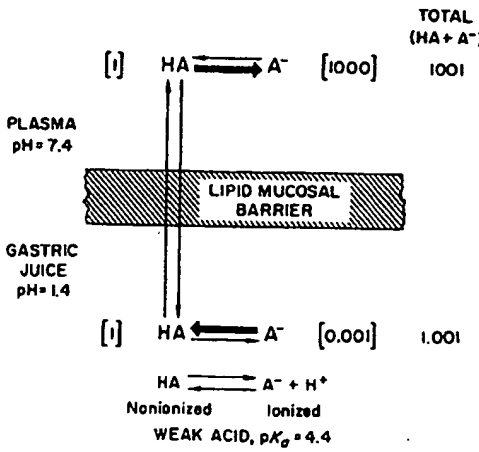


Figure 1–2. *Influence of pH on the distribution of a weak acid between plasma and gastric juice, separated by a lipid barrier.*

to a steady state. For a weak base with a pK_a of 4.4 ($BH^+ \rightleftharpoons B + H^+$), the ratio would be reversed, as would the thick horizontal arrows in Figure 1–2, which indicate the predominant species at each pH. These considerations have obvious implications for the absorption and excretion of drugs, as will be discussed more specifically below. The establishment of concentration gradients of weak electrolytes across membranes with a pH gradient is a purely physical process and does not require an active transport system. All that is necessary is a membrane preferentially permeable to one form of the weak electrolyte and a pH gradient across the membrane. The establishment of the pH gradient is, however, an active process.

Bulk flow through intercellular pores is the major mechanism of passage of drugs across most capillary endothelial membranes, with the important exception of the central nervous system (CNS) (*see* below). These intercellular gaps are sufficiently large that diffusion across most capillaries is limited by blood flow and not by the lipid solubility of drugs or pH gradients. This is an important factor in filtration across glomerular membranes in the kidney (*see* below). Tight junctions are characteristic of capillaries of the CNS and a variety of epithelia. Intercellular diffusion is consequently limited. Pinocytosis, the formation and movement of vesicles across cell membranes, has been implicated in drug absorption. However, the quantitative significance of pinocytosis is questionable.

Carrier-Mediated Membrane Transport. While passive diffusion through the bilayer is dominant in the absorption and distribution of most drugs, more active and selective mechanisms can play important roles. Active transport of some drugs occurs across neuronal membranes, the choroid plexus, renal tubular cells, and hepatocytes. The characteristics of active transport—selectivity, competitive inhibition by congeners, a requirement for energy, saturability, and movement against an electrochemical gradient—may be important in the mechanism of action of drugs that are subject to active transport or that interfere with the active transport of natural metabolites or neurotransmitters. The term *facilitated diffusion* describes a carrier-mediated transport process to which there is no input of energy, and movement of the substance in question thus cannot occur against an electrochemical gradient. Such mechanisms, which may also be highly selective for specific conformational structures of drugs, are necessary for the transport of endogenous compounds whose rate of movement across biological membranes by simple diffusion would otherwise be too slow.

DRUG ABSORPTION, BIOAVAILABILITY, AND ROUTES OF ADMINISTRATION

Absorption describes the rate at which a drug leaves its site of administration and the extent to which this occurs. However, the clinician is primarily concerned with a parameter designated as *bioavailability*, rather than absorption. Bioavailability is a term used to indicate the extent to which a drug reaches its site of action *or* a biological fluid from which the drug has access to its site of action. For example, a drug that is absorbed from the stomach and intestine must first pass through the liver before it reaches the systemic circulation. If the drug is metabolized in the liver or excreted in the bile, some of the active drug will be inactivated or diverted before it can reach the general circulation and be distributed to its sites of action. If the metabolic or excretory capacity of the liver for the agent in question is great, bioavailability will be substantially decreased (the so-called first-pass effect). This decrease in availability is a function of the anatomical site from which absorption takes place; other anatomical, physiological, and pathological factors can influence bioavailability (*see* below), and the choice of the route of drug administration must be based on an understanding of these conditions. Moreover, factors that modify the absorption of a drug can change its bioavailability.

Factors That Modify Absorption. Many variables, in addition to the physicochemical factors that affect transport across membranes, influence the absorption of drugs. Absorption, regardless of the site, is dependent upon drug solubility. Drugs given in aqueous solution are more rapidly absorbed than those given in oily solution, suspension, or solid form because they mix more readily with the aqueous phase at the absorptive site. For those given in solid form, the rate of dissolution may be the lim-

iting factor in their absorption. Local conditions at the site of absorption alter solubility, particularly in the gastrointestinal tract. Aspirin, which is relatively insoluble in acidic gastric contents, is a common example of such a drug. The concentration of a drug influences its rate of absorption. Drugs ingested or injected in solutions of high concentration are absorbed more rapidly than are drugs in solutions of low concentration. The circulation to the site of absorption also affects drug absorption. Increased blood flow, brought about by massage or local application of heat, enhances the rate of drug absorption; decreased blood flow, produced by vasoconstrictor agents, shock, or other disease factors, can slow absorption. The area of the absorbing surface to which a drug is exposed is one of the more important determinants of the rate of drug absorption. Drugs are absorbed very rapidly from large surface areas such as the pulmonary alveolar epithelium, the intestinal mucosa, or, in a few cases after extensive application, the skin. The absorbing surface is determined largely by the route of administration. Each of these factors separately or in conjunction with one another may have profound effects on the efficacy and toxicity of a drug.

Enteral (Oral) vs. Parenteral Administration. Often there is a choice of the route by which a therapeutic agent may be given, and a knowledge of the advantages and disadvantages of the different routes of administration is then of primary importance. Some characteristics of the major routes employed for systemic drug effect are compared in Table 1–1.

Oral ingestion is the most common method of drug administration. It is also the safest, most convenient, and most economical. Disadvantages to the oral route include the incapability to absorb some drugs because of their physical characteristics (*e.g.*, polarity), emesis as a result of irritation to the gastrointestinal mucosa, destruction of some drugs by digestive enzymes or low gastric pH, irregularities in absorption or propulsion in the presence of food or other drugs, and necessity for cooperation on the part of the patient. In addition, drugs in the gastrointestinal tract may be metabolized by the enzymes of the mu-

Table 1–1. SOME CHARACTERISTICS OF COMMON ROUTES OF DRUG ADMINISTRATION *

ROUTE	ABSORPTION PATTERN	SPECIAL UTILITY	LIMITATIONS AND PRECAUTIONS
Intravenous	Absorption circumvented Potentially immediate effects	Valuable for emergency use Permits titration of dosage Suitable for large volumes and for irritating substances, when diluted	Increased risk of adverse effects Must inject solutions *slowly*, as a rule Not suitable for oily solutions or insoluble substances
Subcutaneous	Prompt, from aqueous solution Slow and sustained, from repository preparations	Suitable for some insoluble suspensions and for implantation of solid pellets	Not suitable for large volumes Possible pain or necrosis from irritating substances
Intramuscular	Prompt, from aqueous solution Slow and sustained, from repository preparations	Suitable for moderate volumes, oily vehicles, and some irritating substances	Precluded during anticoagulant medication May interfere with interpretation of certain diagnostic tests (*e.g.*, creatine kinase)
Oral ingestion	Variable; depends upon many factors (*see* text)	Most convenient and economical; usually more safe	Requires patient cooperation Availability potentially erratic and incomplete for drugs that are poorly soluble, slowly absorbed, unstable, or extensively metabolized by the liver

* *See* text for more complete discussion and for other routes.

cosa, the intestinal flora, or the liver before they gain access to the general circulation.

The parenteral injection of drugs has certain distinct advantages over oral administration. In some instances, parenteral administration is essential for the drug to be absorbed in active form. Availability is usually more rapid and more predictable than when a drug is given by mouth. The effective dose can therefore be more accurately selected. In emergency therapy, parenteral administration is particularly serviceable. If a patient is unconscious, uncooperative, or unable to retain anything given by mouth, parenteral therapy may be a necessity. The injection of drugs also has its disadvantages. Asepsis must be maintained, an intravascular injection may occur when it is not intended, pain may accompany the injection, and it is sometimes difficult for a patient to perform the injection himself if self-medication is necessary. Expense is another consideration.

Oral Ingestion. Absorption from the gastrointestinal tract is governed by factors that are generally applicable, such as surface area for absorption, blood flow to the site of absorption, the physical state of the drug, and its concentration at the site of absorption. Since most drug absorption from the gastrointestinal tract occurs via passive processes, absorption is favored when the drug is in the nonionized and more lipophilic form. Thus, one might expect the absorption of weak acids to be optimal in the acidic environment of the stomach, whereas absorption of bases might be favored in the relatively alkaline small intestine. However, it is an oversimplification to extrapolate the pH-partition concept presented in Figure 1–2 to a comparison of two different biological membranes, such as the epithelia of the stomach and the intestine. The stomach is lined by a thick, mucus-covered membrane with a small surface area and high electrical resistance. The primary function of the stomach is digestive. In contrast, the epithelium of the intestine has an extremely large surface area; it is thin, it has low electrical resistance, and its primary function is to facilitate the absorption of nutrients. Thus, any factor that accelerates gastric emptying will be likely to increase the rate of drug absorption, while any factor that delays gastric emptying will probably have the opposite effect, regardless of the characteristics of the drug. The experimental data available from the classical work of Brodie (1964) and more recent studies (Prescott and Nimmo, 1981) are all consistent with the following conclusion: the nonionized form of a drug will be absorbed more rapidly than the ionized form at any particular site in the gastrointestinal tract. However, the rate of absorption of a drug from the intestine will be greater than that from the stomach even if the drug is predominantly ionized in the intestine and largely nonionized in the stomach.

Drugs that are destroyed by gastric juice or that cause gastric irritation are sometimes administered in dosage forms with a coating that prevents dissolution in the acidic gastric contents. However, some enteric-coated preparations of a drug also may resist dissolution in the intestine, and very little of the drug may be absorbed.

Controlled-Release Preparations. The rate of absorption of a drug administered as a tablet or other solid oral-dosage form is partly dependent upon its rate of dissolution in the gastrointestinal fluids. This factor is the basis for the so-called controlled-release, timed-release, sustained-release, or prolonged-action pharmaceutical preparations that are designed to produce slow, uniform absorption of the drug for 8 hours or longer. Potential advantages of such preparations are reduction in the frequency of administration of the drug as compared with conventional dosage forms (possibly with improved compliance by the patient), maintenance of a therapeutic effect overnight, and decreased incidence and/or intensity of undesired effects by elimination of the peaks in drug concentration that often occur after administration of immediate-release dosage forms.

Many controlled-release preparations fulfill these theoretical expectations. However, the clinician must be aware of some drawbacks of these products. Generally, interpatient variability in terms of the systemic concentration of the drug that is achieved is greater for controlled-release as compared with immediate-release dosage forms. During repeated drug administration, trough drug concentrations resulting from controlled-release dosage forms may not be different from those observed with immediate-release preparations, although the time interval between trough concentrations is greater for a well-designed controlled-release product. It is possible that the dosage form may fail, and "dose-dumping" with resultant toxicity can occur, since the total dose of

drug ingested at one time may be several times the amount contained in the conventional preparation. Controlled-release dosage forms are most appropriate for drugs with short half-lives (less than 4 hours). So-called controlled-release dosage forms are sometimes developed for drugs with long half-lives (greater than 12 hours). These usually more expensive products should not be prescribed unless specific advantages have been demonstrated.

Sublingual Administration. Absorption from the oral mucosa has special significance for certain drugs, despite the fact that the surface area available is small. For example, nitroglycerin is effective when retained sublingually because it is nonionic and has a very high lipid solubility. Thus, the drug is absorbed very rapidly. Nitroglycerin is also very potent; relatively few molecules need to be absorbed to produce the therapeutic effect. Since venous drainage from the mouth is to the superior vena cava, the drug is also protected from rapid first-pass metabolism by the liver. Hepatic first-pass metabolism is sufficient to prevent the appearance of any active nitroglycerin in the systemic circulation if the conventional tablet is swallowed.

Rectal Administration. The rectal route is often useful when oral ingestion is precluded by vomiting or when the patient is unconscious. Approximately 50% of the drug that is absorbed from the rectum will bypass the liver; the potential for hepatic first-pass metabolism is thus less than that for an oral dose. However, rectal absorption is often irregular and incomplete, and many drugs cause irritation of the rectal mucosa.

Parenteral Injection. The major routes of parenteral administration are intravenous, subcutaneous, and intramuscular. Absorption from subcutaneous and intramuscular sites occurs by simple diffusion along the gradient from drug depot to plasma. The rate is limited by the area of the absorbing capillary membranes and by the solubility of the substance in the interstitial fluid. Relatively large aqueous channels in the endothelial membrane account for the indiscriminate diffusion of molecules regardless of their lipid solubility. Larger molecules, such as proteins, slowly gain access to the circulation by way of lymphatic channels.

Drugs administered into the systemic circulation by any route, excluding the intra-arterial, are subject to possible first-pass elimination in the lung prior to distribution to the rest of the body. The lungs serve as a temporary clearing site for a number of agents, especially drugs that are weak bases and are predominantly nonionized at the blood pH, apparently by their partition into lipid. The lungs also serve as a filter for particulate matter that may be given intravenously, and, of course, they provide a route of elimination for volatile substances.

Intravenous. The factors concerned in absorption are circumvented by intravenous injection of drugs in aqueous solution, and the desired concentration of a drug in blood is obtained with an accuracy and immediacy not possible by any other procedure. In some instances, as in the induction of surgical anesthesia by a barbiturate, the dose of a drug is not predetermined but is adjusted to the response of the patient. Also, certain irritating solutions can be given only in this manner, since the blood vessel walls are relatively insensitive and the drug, if injected slowly, is greatly diluted by the blood.

As there are assets to the use of this route of administration, so are there liabilities. Unfavorable reactions are likely to occur, since high concentrations of drug may be attained rapidly in both plasma and tissues. Once the drug is injected there is no retreat. Repeated intravenous injections are dependent upon the ability to maintain a patent vein. Drugs in an oily vehicle or those that precipitate blood constituents or hemolyze erythrocytes should not be given by this route. Intravenous injection must usually be performed slowly and with constant monitoring of the responses of the patient.

Subcutaneous. Injection of a drug into a subcutaneous site is often used. It can be used only for drugs that are not irritating to tissue; otherwise, severe pain, necrosis, and slough may occur. The rate of absorption following subcutaneous injection of a drug is often sufficiently constant and slow to provide a sustained effect. Moreover, it may be varied intentionally. For example, the rate of absorption of a suspension of insoluble insulin is slow compared with that of a soluble preparation of the hormone. The incorporation of a vasoconstrictor agent in a solution of a drug to be injected subcutaneously also retards absorption. Absorption of drugs implanted under the skin in a solid pellet form occurs slowly over a period of weeks or months; some hormones are effectively administered in this manner.

Intramuscular. Drugs in aqueous solution are absorbed quite rapidly after intramuscular injection, depending upon the rate of blood flow to the injection site. Joggers who inject insulin into their thigh may experience a precipitous drop in blood sugar that is not seen following injection into the arm or abdominal wall, since running markedly increases blood flow to the leg. Generally, the rate of absorption following injection of an aqueous preparation into the deltoid or vastus lateralis is faster than when the injection is made into the gluteus maximus. The rate is particularly slower for females after injection into the gluteus maximus. This has been attributed to the different distribution of subcutaneous fat in males and females, since fat is relatively poorly perfused. Very obese or emaciated patients may exhibit unusual patterns of absorption following intramuscular or subcutaneous injection. Very slow, constant absorption from the intramuscular site results if the drug is injected in solution in oil or suspended in various other repository vehicles. Penicillin is often administered in this manner. Substances too irritating to be injected subcutaneously may sometimes be given intramuscularly.

Intra-arterial. Occasionally a drug is injected directly into an artery to localize its effect in a particular tissue or organ. However, this practice usually has dubious therapeutic value. Diagnostic agents are sometimes administered by this route. Intra-arterial injection requires great care and should be reserved for experts. The first-pass and cleansing effects of the lung are not available when drugs are given by this route.

Intrathecal. The blood–brain barrier and the blood–cerebrospinal fluid barrier often preclude or slow the entrance of drugs into the CNS. Therefore, when local and rapid effects of drugs on the meninges or cerebrospinal axis are desired, as in spinal anesthesia or acute CNS infections, drugs are sometimes injected directly into the spinal subarachnoid space.

Intraperitoneal. The peritoneal cavity offers a large absorbing surface from which drugs enter the circulation rapidly, but primarily by way of the portal vein; first-pass hepatic losses are thus possible. Intraperitoneal injection is a common laboratory procedure, but it is seldom employed clinically. The dangers of producing infection and adhesions are too great to warrant the routine use of this route in man.

Pulmonary Absorption. Gaseous and volatile drugs may be inhaled and absorbed through the pulmonary epithelium and mucous membranes of the respiratory tract. Access to the circulation is rapid by this route, because the surface area is large. The principles governing absorption and excretion of the anesthetic gases and vapors are discussed in Chapter 13.

In addition, solutions of drugs can be atomized and the fine droplets in air (aerosol) inhaled. Advantages are the almost instantaneous absorption of a drug into the blood, avoidance of hepatic first-pass loss, and, in the case of pulmonary disease, local application of the drug at the desired site of action. For example, β-adrenergic agonists can be given in this manner for the treatment of bronchial asthma. The main disadvantages are poor ability to regulate the dose, cumbersomeness of the methods of administration, and the fact that many gaseous and volatile drugs produce irritation of the pulmonary epithelium.

Pulmonary absorption is an important route of entry of certain drugs of abuse and of toxic environmental substances of varied composition and physical states (*see* Section XVIII). Both local and systemic reactions to allergens may occur subsequent to inhalation.

Topical Application. *Mucous Membranes.* Drugs are applied to the mucous membranes of the conjunctiva, nasopharynx, oropharynx, vagina, colon, urethra, and urinary bladder primarily for their local effects. Occasionally, as in the application of antidiuretic hormone to the nasal mucosa, systemic absorption is the goal. Absorption through mucous membranes occurs readily. In fact, local anesthetics applied for local effect may sometimes be absorbed so rapidly that they produce systemic toxicity.

Skin. Few drugs readily penetrate the intact skin. Absorption of those that do is proportional to the surface area over which they are applied and to their lipid solubility, since the epidermis behaves as a lipid barrier. The dermis, however, is freely permeable to many solutes; consequently, systemic absorption of drugs occurs much more readily through abraded, burned, or denuded skin. Inflammation and other conditions that increase cutaneous blood flow also enhance absorption. Toxic effects are sometimes produced by absorption through the skin of highly lipid-soluble substances (*e.g.*, a lipid-soluble insecticide in an organic solvent). Absorption through the skin can be enhanced by suspending the drug in an oily vehicle and rubbing the resulting preparation into the skin. This method of administration is known as inunction. Because hydrated skin is more permeable than dry skin, the dosage form may be modified or an occlusive dressing may be used to facilitate absorption. Controlled-release topical patches are recent innovations. A patch containing scopolamine, placed behind the ear where body temperature and blood flow enhance absorption, releases sufficient drug to the systemic circulation to protect the wearer from motion sickness. Patches containing nitroglycerin are used to provide sustained delivery of a drug that is subject to extensive first-

pass metabolism after oral administration (*see* Ridout *et al.*, 1988).

Eye. Topically applied ophthalmic drugs are used primarily for their local effects. Systemic absorption that results from drainage through the nasolacrimal canal is usually undesirable. In addition, drug that is absorbed after such drainage is not subject to first-pass hepatic elimination. Unwanted systemic pharmacological effects may occur for this reason when β-adrenergic antagonists are administered as ophthalmic drops. Local effects usually require absorption of the drug through the cornea; corneal infection or trauma may thus result in more rapid absorption. Ophthalmic delivery systems that provide prolonged duration of action (*e.g.*, suspensions and ointments) are useful additions to ophthalmic therapy. Ocular inserts, developed more recently, provide continuous delivery of low amounts of drug. Very little is lost through drainage; hence, systemic side effects are minimized.

Bioequivalence. Drug products are considered to be pharmaceutical equivalents if they contain the same active ingredients and are identical in strength or concentration, dosage form, and route of administration. Two pharmaceutically equivalent drug products are considered to be bioequivalent when the rates and extents of bioavailability of the active ingredient in the two products are not significantly different under suitable test conditions. In the past, dosage forms of a drug from different manufacturers and even different lots of preparations from a single manufacturer sometimes differed in their bioavailability. Such differences were seen primarily among oral dosage forms of poorly soluble, slowly absorbed drugs. They result from differences in crystal form, particle size, or other physical characteristics of the drug that are not rigidly controlled in formulation and manufacture of the preparations. These factors affect disintegration of the dosage form and dissolution of the drug and hence the rate and extent of drug absorption.

The potential nonequivalence of different drug preparations is a matter of concern. Strengthened regulatory requirements over the past few years have resulted in significantly fewer documented cases of nonequivalence between approved drug products. However, since equivalence of measured systemic concentrations of active drug and known metabolites is not necessarily proof of therapeutic equivalence, some clinicians prefer to maintain certain "fragile" patients on a single manufacturer's product. The significance of possible nonequivalence of drug preparations is further discussed in connection with drug nomenclature and the choice of drug name in writing prescription orders (*see* Appendix I).

DISTRIBUTION OF DRUGS

After a drug is absorbed or injected into the bloodstream, it may be distributed into interstitial and cellular fluids. Patterns of drug distribution reflect certain physiological factors and physicochemical properties of drugs. An initial phase of distribution may be distinguished that reflects cardiac output and regional blood flow. Heart, liver, kidney, brain, and other well perfused organs receive most of the drug during the first few minutes after absorption. Delivery of drug to muscle, most viscera, skin, and fat is slower, and these tissues may require several minutes to several hours before steady state is attained. A second phase of drug distribution may therefore be distinguished; this is also limited by blood flow, and it involves a far larger fraction of the body mass than does the first phase. Superimposed on patterns of distribution of blood flow are factors that determine the rate at which drugs diffuse into tissues. Diffusion into the interstitial compartment occurs rapidly because of the highly permeable nature of capillary endothelial membranes (except in the brain). Lipid-insoluble drugs that permeate membranes poorly are restricted in their distribution and hence in their potential sites of action. Distribution may also be limited by drug binding to plasma proteins, particularly albumin for acidic drugs and α_1-acid glycoprotein for basic drugs. An agent that is extensively and strongly bound has limited access to cellular sites of action, and it may be metabolized and eliminated slowly. Drugs may accumulate in tissues in higher concentrations than would be expected from diffusion equilibria as a result of pH gradients, binding to intracellular constituents, or partitioning into lipid.

Drug that has accumulated in a given tissue may serve as a reservoir that prolongs drug action in that same tissue or at a distant site reached through the circulation. An example that illustrates many of these factors is the use of the intravenous anesthetic thiopental, a highly lipid-soluble drug. Because blood flow to the brain is so high, the drug reaches its maximal concentration in brain within a minute after it is injected intravenously. After injection is concluded, the plasma concentration falls as thiopental diffuses into other tissues, such as muscle. The concentration of the drug in brain follows that of the plasma,

because there is little binding of the drug to brain constituents. Thus, onset of anesthesia is rapid, but so is its termination. Both are directly related to the concentration of drug in the brain. A third phase of distribution for this drug is due to the slow, blood-flow–limited uptake by fat. With administration of successive doses of thiopental, accumulation of drug takes place in fat and other tissues that can store large amounts of the compound. These can become reservoirs for the maintenance of the plasma concentration, and, therefore the brain concentration, at or above the threshold required for anesthesia. Thus, a drug that is short acting because of rapid redistribution to sites at which the agent has no pharmacological action can become long acting when these storage sites are "filled" and termination of the drug's action becomes dependent on biotransformation and excretion (*see* Benet, 1978).

Since the difference in pH between intracellular and extracellular fluids is small (7.0 vs. 7.4), this factor can result in only a relatively small concentration gradient of drug across the plasma membrane. Weak bases are concentrated slightly inside of cells, while the concentration of weak acids is slightly lower in the cells than in extracellular fluids. Lowering the pH of extracellular fluid increases the intracellular concentration of weak acids and decreases that of weak bases, provided that the intracellular pH does not also change and that the pH change does not simultaneously affect the binding, biotransformation, or excretion of the drug. Elevating the pH produces the opposite effects (*see* Figure 1–2).

Central Nervous System and Cerebrospinal Fluid. The distribution of drugs to the CNS from the bloodstream is unique, mainly in that entry of drugs into the cerebrospinal fluid and extracellular space of the CNS is restricted. The restriction is similar to that across the gastrointestinal epithelium. Endothelial cells of the brain capillaries differ from their counterparts in most tissues by the absence of intercellular pores and pinocytotic vesicles. Tight junctions predominate, and aqueous bulk flow is thus severely restricted. This is not unique to the CNS capillaries (tight junctions appear in many muscle capillaries as well). It is likely that the unique arrangement of pericapillary glial cells also contributes to the slow diffusion of organic acids and bases into the CNS. The drug molecules probably must traverse not only endothelial but also perivascular cell membranes before reaching neurons or other target cells in the CNS. Cerebral blood flow is the only limitation to permeation of the CNS by highly lipid-soluble drugs. With increasing polarity the rate of diffusion of drugs into the CNS is proportional to the lipid solubility of the nonionized species. Strongly ionized agents such as quaternary amines are normally unable to enter the CNS from the circulation.

In addition, organic ions are extruded from the cerebrospinal fluid into blood at the choroid plexus by transport processes similar to those in the renal tubule. Lipid-soluble substances leave the brain by diffusion through the capillaries and the blood–choroid plexus boundary. Drugs and endogenous metabolites, regardless of lipid solubility and molecular size, also exit with bulk flow of the cerebrospinal fluid through the arachnoid villi.

The blood–brain barrier is adaptive in that exclusion of drugs and other foreign agents such as penicillin or tubocurarine protects the CNS against severely toxic effects. However, the barrier is neither absolute nor invariable. Very large doses of penicillin may produce seizures; meningeal or encephalic inflammation increases the local permeability. Maneuvers to increase permeability of the blood–brain barrier are potentially important to enhance the efficacy of chemotherapeutic agents that are used to treat infections or tumors localized in the brain.

Drug Reservoirs. As mentioned, the body compartments in which a drug accumulates are potential reservoirs for the drug. If stored drug is in equilibrium with that in plasma and is released as the plasma concentration declines, a concentration of the drug in plasma and at its locus of action is sustained, and pharmacological effects of the drug are prolonged. However, if the reservoir for the drug has a large capacity and fills rapidly, it so alters the distribution of the drug that larger quantities of the drug are required initially to provide a therapeutically effective concentration in the target organ.

Plasma Proteins. Many drugs are bound to plasma proteins, mostly to plasma albumin for acidic drugs and to α_1-acid glycoprotein for basic drugs; binding to other plasma proteins generally occurs to a much smaller extent. The binding is usually reversible; covalent binding of reactive drugs

such as alkylating agents occurs occasionally.

The fraction of total drug in plasma that is bound is determined by the drug concentration, its affinity for the binding sites, and the number of binding sites. Simple mass-action equations are used to describe the free and bound concentrations (*see* Chapter 2). At low concentrations of drug (less than the plasma protein–binding dissociation constant), the fraction bound is a function of the concentration of binding sites and the dissociation constant. At high drug concentrations (greater than the dissociation constant), the fraction bound is a function of the number of binding sites and the drug concentration. Therefore, statements that a given drug is bound to a specified extent apply only over a limited range of concentrations. The percentage values listed in Appendix II refer only to the therapeutic range of concentrations for each drug.

Binding of a drug to plasma proteins limits its concentration in tissues and at its locus of action, since only unbound drug is in equilibrium across membranes. Binding also limits glomerular filtration of the drug, since this process does not immediately change the concentration of free drug in the plasma (water is also filtered). However, plasma protein binding does *not* generally limit renal tubular secretion or biotransformation, since these processes lower the free drug concentration, and this is rapidly followed by dissociation of the drug–protein complex. If a drug is avidly transported or metabolized and its clearance, calculated on the basis of unbound drug, exceeds organ plasma flow, binding of the drug to plasma protein may be viewed as a transport mechanism that fosters drug elimination by delivering drug to sites for elimination.

Since binding of drugs to plasma proteins is rather nonselective, many drugs with similar physicochemical characteristics can compete with each other and with endogenous substances for these binding sites. For example, displacement of unconjugated bilirubin from binding to albumin by the sulfonamides and other organic anions is known to increase the risk of bilirubin encephalopathy in the newborn, and drug toxicity has sometimes been attributed to similar competition between drugs for binding sites. Such interactions are often more complex than generally stated. Since drug displaced from plasma protein will redistribute into its full potential volume of distribution, the concentration of free drug in plasma and tissues after redistribution may be increased only slightly. The interaction may also involve altered elimination of the drug. Risk of adverse effect is greatest if the displaced drug has a limited volume of distribution, if the competition extends to the drug bound in tissues, if elimination of the drug is also reduced, or if the displacing drug is administered in high dosage by rapid intravenous injection. Competition of drugs for plasma protein-binding sites may also cause misinterpretation of measured concentrations of drugs in plasma, since most assays do not distinguish free from bound drug.

Cellular Reservoirs. Many drugs accumulate in muscle and other cells in higher concentrations than in the extracellular fluids. If the intracellular concentration is high and if the binding is reversible, the tissue involved may represent a sizable drug reservoir, particularly if the tissue represents a large fraction of body mass. For example, during long-term administration of the antimalarial agent quinacrine, the concentration of the drug in liver may be several thousand times that in plasma. Accumulation in cells may be the result of active transport or, more commonly, binding. Tissue binding of drugs usually occurs to proteins, phospholipids, or nucleoproteins and is generally reversible.

Fat as a Reservoir. Many lipid-soluble drugs are stored by physical solution in the neutral fat. In obese persons, the fat content of the body may be as high as 50%, and even in starvation it constitutes 10% of body weight; hence, fat can serve as an important reservoir for lipid-soluble drugs. For example, as much as 70% of the highly lipid-soluble barbiturate thiopental may be present in body fat 3 hours after administration. However, fat is a rather stable reservoir because it has a relatively low blood flow.

Bone. The tetracycline antibiotics (and other divalent-metal-ion chelating agents) and heavy metals may accumulate in bone by adsorption onto the bone-crystal surface and eventual incorporation into the crystal lattice. Bone can become a reservoir for the slow release of toxic agents such as lead or radium into the blood. Their effects can

thus persist long after exposure has ceased. Local destruction of the bone medulla may also lead to reduced blood flow and prolongation of the reservoir effect, since the toxic agent becomes sealed off from the circulation; this may further enhance the direct local damage to the bone. A vicious cycle results whereby the greater the exposure to the toxic agent the slower is its rate of elimination.

Transcellular Reservoirs. Drugs also cross epithelial cells and may accumulate in the transcellular fluids. The major transcellular reservoir is the gastrointestinal tract. Weak bases are passively concentrated in the stomach from the blood, because of the large pH differential between the two fluids, and some drugs are secreted in the bile in an active form or as a conjugate that can be hydrolyzed in the intestine. In these cases, and when an orally administered drug is slowly absorbed, the gastrointestinal tract serves as a drug reservoir.

Other transcellular fluids, including cerebrospinal fluid, aqueous humor, endolymph, and joint fluids, do not generally accumulate significant total amounts of drugs.

Redistribution. Termination of drug effect is usually by biotransformation and excretion, but it may also result from redistribution of the drug from its site of action into other tissues or sites. Redistribution is a factor in terminating drug effect primarily when a highly lipid-soluble drug that acts on the brain or cardiovascular system is administered rapidly by intravenous injection or by inhalation. The factors involved in redistribution of drugs have been discussed above.

Placental Transfer of Drugs. The potential transfer of drugs across the placenta is important, since drugs may cause congenital anomalies. Administered immediately before delivery, they may also have adverse effects on the neonate. Drugs cross the placenta primarily by simple diffusion. Lipid-soluble, nonionized drugs readily enter the fetal blood from the maternal circulation. Penetration is least with drugs possessing a high degree of dissociation or low lipid solubility. The view that the placenta is a barrier to drugs is inaccurate. A more appropriate approximation is that the fetus is to at least some extent exposed to essentially all drugs taken by the mother.

BIOTRANSFORMATION OF DRUGS

The physicochemical properties of drug molecules that permit rapid passage across cellular membranes during absorption and distribution also impair subsequent excretion. For example, after filtration at the renal glomerulus most lipid-soluble drugs largely escape excretion from the body because they are readily reabsorbed from the filtrate by diffusion through the renal tubular cells. Thus, the enzymatic biotransformation of drugs to more polar and less lipid-soluble metabolites enhances their excretion and reduces their volume of distribution. Such biotransformation relieves the burden of foreign chemicals and is critical for the survival of the organism. Studies of the genes that encode the enzymes of biotransformation have led to the view that they evolved millions of years ago as a mechanism for removal of natural constituents of foods, such as flavones, terpenes, steroids, and alkaloids. (For excellent summaries of drug biotransformation, *see* Goldstein *et al.*, 1974; Lee *et al.*, 1977; Jacqz *et al.*, 1986; Nebert and Gonzalez, 1987.)

Enzymes Responsible for Biotransformation. The enzyme systems responsible for the biotransformation of many drugs are located in the smooth endoplasmic reticulum of the liver (operationally designated the microsomal fraction). These enzymes also are present in other organs, such as the kidney, lung, and gastrointestinal epithelium, although in smaller quantities. Drugs absorbed from the intestine may thus be subject to the first-pass effect. This represents the combined action of hepatic and gastrointestinal epithelial enzymes, which can at times prevent effective concentrations of active drug from reaching the systemic circulation after oral administration, as discussed above.

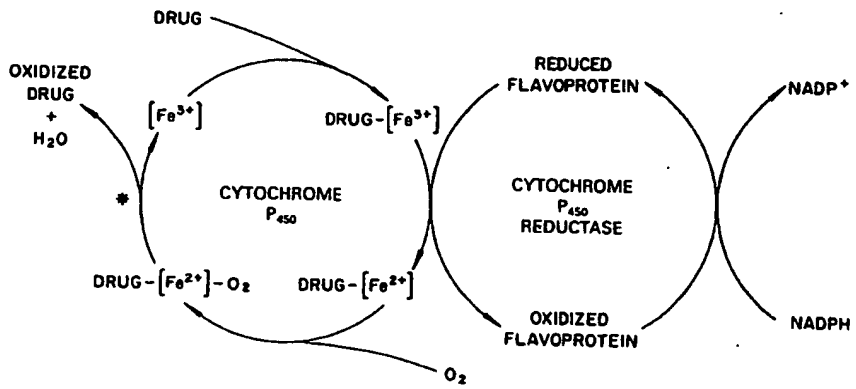
The chemical reactions of enzymatic biotransformation are classified as either phase-I or phase-II reactions. Phase-I reactions convert the parent drug to a more polar metabolite by oxidation, reduction, or hydrolysis. The resulting metabolite may be pharmacologically inactive, less active, or occasionally more active than the parent molecule. When the metabolite itself is the active drug, the parent compound is said to be a *prodrug* (*e.g.*, enalapril). Phase-II reactions, which are also called conjugation

out by a large family of isozymes called cytochromes P_{450} (Figure 1–3). The cytochrome P_{450} proteins are embedded in the lipid bilayer of the smooth endoplasmic reticulum. An important associated protein, NADPH–cytochrome P_{450} oxidoreductase, is also attached to this lipid bilayer in a stoichiometry of about ten P_{450} molecules to one reductase. A drug substrate initially binds to oxidized (Fe^{3+}) cytochrome P_{450} (Figure 1–3). The resulting drug-cytochrome complex is reduced by the reductase, and the reduced complex then combines with molecular oxygen. A second electron and two hydrogen ions are acquired from the donor system, and the subsequent products are oxidized metabolite and water, with regeneration of the oxidized cytochrome P_{450}. As mentioned, the cytochromes P_{450} are the dominant phase-I oxidative system, although oxidation by amine oxidases, several heme peroxidases, prostaglandin H synthase, xanthine oxidase, and alcohol and aldehyde dehydrogenases are also important.

Hydrolysis. Esters such as procaine (*see* Table 1–2) are hydrolyzed by a variety of nonspecific esterases in liver, plasma, the gastrointestinal tract, and other tissues. Hydrolysis of amides, such as lidocaine, occurs primarily in the liver. Proteases and peptidases in plasma, erythrocytes, and many other tissues are involved in the biotransformation of polypeptide drugs. With the marked interest in the therapeutic application of proteins and peptides, these enzymatic reactions have assumed greater importance. Delivery of such drugs across biological membranes requires the inhibition of these enzymes or the masking of their substrates.

Reduction. Enzymes in the endoplasmic reticulum and cytosol of liver and other tissues can catalyze the reduction of nitro groups (*e.g.*, chloramphenicol) and the cleavage and reduction of an azo linkage (*e.g.*, prontosil) (*see* Table 1–2).

Conjugations. The formation of glucuronides is catalyzed by hepatic glucuronyltransferases, located in the endoplasmic reticulum; these enzymes use uridine diphosphate-glucuronate as the donor of glucuronate. Conjugation with glucuronate also occurs in the kidney and other tissues, but to a much lesser extent. Glucuronides constitute the major proportion of metabolites of many drugs that contain a phenol, alcohol, or carboxylate group (*see* Table 1–2). They are generally inactive and are rapidly secreted into the urine and bile by an anion transport system. However, concentrations of some glucuronides in plasma may approach those of the parent compound. Glucuronides formed from carboxylic acids (*i.e.*, ester glucuronides) are readily hydrolyzed back to the parent compound (both enzymatically and spontaneously). Glucuronides formed from phe-



* Denotes contribution of a second electron and two hydrogen ions from NADH-flavoprotein-cytochrome b_5 or from NADPH-flavoprotein.

Figure 1–3. *Major components of the hepatic cytochrome P_{450} monooxygenase system.*

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nols or alcohols (*i.e.*, ether glucuronides) are stable chemically. However, both types of glucuronide can be hydrolyzed enzymatically in the intestine following biliary excretion, with subsequent reabsorption of the liberated drug. This enterohepatic cycling may prolong the action of the drug.

Conjugation of aromatic primary amines or hydrazines with acetic acid (using acetyl coenzyme A as the acetyl donor) involves several N-acetyl transferases. Examples include many sulfonamides and drugs such as isoniazid, hydralazine, and procainamide. Aromatic carboxylic acids, such as salicylic acid, are often inactivated by conjugation with glycine. Other reactions include conjugation of phenolic compounds (including steroids) with sulfate and O-, S-, and N-methylation of amines and phenols (*e.g.*, epinephrine). Conjugation with the nucleophilic sulfhydryl-containing tripeptide, glutathione, is not usually quantitatively significant (and is not shown in Table 1–2). However, it contributes to inactivation of unstable and potentially toxic intermediates produced during some biotransformation reactions and thus is important (*see* below).

Factors That Modify Biotransformation. Studies of biotransformation in experimental animals have shown a large number of genetic, environmental, and physiological factors that affect the metabolic fate of a drug. In man, the most important factors are genetically determined polymorphisms in drug oxidations and conjugations; environmental influences, including concomitant use of other drugs that induce or inhibit drug-metabolizing enzymes; and the presence of liver disease, often with severe malnutrition.

At least ten families of cytochrome P_{450} genes are now known to constitute the P_{450} gene superfamily (*see* Nebert and Gonzalez, 1987). The ancestral cytochrome P_{450} gene was probably present more than 1.5 billion years ago. It is believed that some of the P_{450} gene families evolved and diverged because of exposure of organisms to plant metabolites and decayed plant products; this has led to the remarkable overlap of substrate specificities of the P_{450} enzymes. Numerous examples are known of drugs that are good substrates for the enzymes encoded by two or more P_{450} gene families that diverged so long ago as to be unlinked chromosomally. It is speculated that P_{450}s in certain other families are more closely related to the earliest P_{450} and carry out critical metabolic transformations of endogenous substances including steroid biosynthesis and fatty acid catabolism.

At this time the basis of genetic polymorphism, the number of cytochrome P_{450} isozymes, the extent of microheterogeneity of these isozymes, and the mechanisms that regulate gene expression have not been examined in sufficient detail for complete delineation (*see* Bridges, 1987). However, two major types of P_{450} enzyme inducers have been identified—aromatic hydrocarbons and agents that resemble phenobarbital. A number of other inducers of individual isozymes also have been detected (*e.g.*, ethanol, rifampin, dexamethasone, clofibrate). Inducers of the phenobarbital type enhance accumulation of biotransforming enzymes in selected organs (liver, intestine), whereas the aromatic hydrocarbons induce enzymes in most tissues. Moreover, just as subsets of P_{450} enzymes are induced by various chemicals, subsets of glucuronyltransferases and other enzymes are also induced by these same agents.

In man, inhibition of the P_{450} enzymes also occurs commonly after exposure to two drugs, cimetidine and ethanol. In addition, competitive inhibition between the many substrates for the enzymes of biotransformation is readily demonstrated *in vitro*. Such interactions are not usually of practical significance *in vivo*. This is not unexpected, since the inactivation of most drugs *in vivo* exhibits first-order rather than zero-order kinetics; that is, the activity of the enzymes is usually not rate limiting. Drug concentrations are commonly well below those necessary to saturate metabolizing enzymes, and competition between substrates is minimized under these conditions. An important corollary, however, is that significant mutual inhibition of drug metabolism is to be expected for drugs that normally exhibit saturable inactivation kinetics. For example, dicumarol inhibits the metabolism of phenytoin and can increase the incidence and severity of side effects of phenytoin, such as ataxia and drowsiness.

Reduction in the rate of drug metabolism may also occur when biotransformation is so rapid that hepatic blood flow is the rate-limiting factor. Hepatic blood flow may decrease acutely after β-adrenergic blockade, and this can affect the rate of metabolism of drugs that are cleared from the plasma at very high rates (*e.g.*, lidocaine). Certain drugs can inhibit the activity of cytochrome P_{450} irreversibly because of covalent interaction with a reactive intermediate generated by the enzyme.

Biotransformation in the Fetus and Neonate. The activities of the hepatic biotransformation enzymes are low in the neonate, particularly in premature babies. Reduced conjugating activity contributes to hyperbilirubinemia and the risk of bilirubin-induced encephalopathy. It is also the basis for the increased toxicity in the neonate of

drugs such as chloramphenicol and certain opioids. A poorly developed blood–brain barrier, weak biotransformation activity, and immature mechanisms for excretion combine to make the fetus and neonate very vulnerable to toxic effects of drugs. The capacity for biotransformation increases during the early months of postnatal life, although the pattern for different enzymes is variable. Cytochrome P_{450} enzymes are usually near adult activities after a few months, whereas phase-II enzymes develop more slowly.

Biotransformation in the Elderly. It is more difficult to generalize about the effect of advanced age on the biotransformation of drugs; the elderly are in many ways a more heterogeneous group than the very young. Because of wide differences in the rates of deterioration of enzyme systems and organs of elimination with age, it is impossible to make blanket recommendations for adjustments of dosage in the elderly. Although the metabolism of some drugs (*e.g.*, quinidine) may be decreased, there are no changes in rates of metabolic clearance of most drugs with age. However, because of the heterogeneity of the elderly population, a small subset of patients may experience alterations in biotransformation, and they may not be identified by studies conducted in healthy, elderly normal volunteers.

Relationship of Biotransformation to Drug Toxicity. Reference was made above to the formation of unstable and potentially toxic metabolites as the result of biotransformation of drugs. Oxidation can cause the formation of highly reactive compounds that normally have such a transient existence that they exert no biological action. Two examples of formation and inactivation of such substances are shown in Figure 1–4. As long as the terminal hydroxylation or conjugation keeps pace, accumulation of reactive intermediates does not occur. However, when induction occurs or when very large amounts of drug are present, oxidation by cytochrome P_{450} is accelerated. Since glutathione is in limited supply in liver and kidney and can be depleted, the drug epoxide or quinone may reach a sufficient concentration to react with nucleophilic cell constituents rather than with glutathione. Hepatic or renal necrosis results. The discovery that the availability of glutathione determines the threshold for the toxic response has led to attempts to use thiols (*e.g.*, N-acetylcysteine) to treat poisoning by drugs such as acetaminophen.

EXCRETION OF DRUGS

Drugs are eliminated from the body either unchanged or as metabolites. Excretory organs, the lung excluded, eliminate polar compounds more efficiently than substances with high lipid solubility. Lipid-soluble drugs are thus not readily eliminated until they are metabolized to more polar compounds.

The kidney is the most important organ for elimination of drugs and their metabolites. Substances excreted in the feces are mainly unabsorbed orally ingested drugs or metabolites excreted in the bile and not reabsorbed from the intestinal tract. Excretion of drugs in breast milk is important not because of the amounts eliminated but because the excreted drugs are potential sources of unwanted pharmacological effects in the nursing infant. Pulmonary excretion is important mainly for the elimination of anesthetic gases and vapors (*see* Chapter 13); occasionally, small quantities of other drugs or metabolites are excreted by this route.

Renal Excretion. Excretion of drugs and metabolites in the urine involves three processes: glomerular filtration, active tubular secretion, and passive tubular reabsorption.

The amount of drug entering the tubular lumen by filtration is dependent on its fractional plasma protein binding and glomerular filtration rate. In the proximal renal tubule, certain organic anions and cations are added to the glomerular filtrate by active, carrier-mediated tubular secretion. Many organic acids (such as penicillin) and metabolites (such as glucuronides) are transported by the system that secretes naturally occurring substances such as uric acid; organic bases, such as tetraethylammonium,

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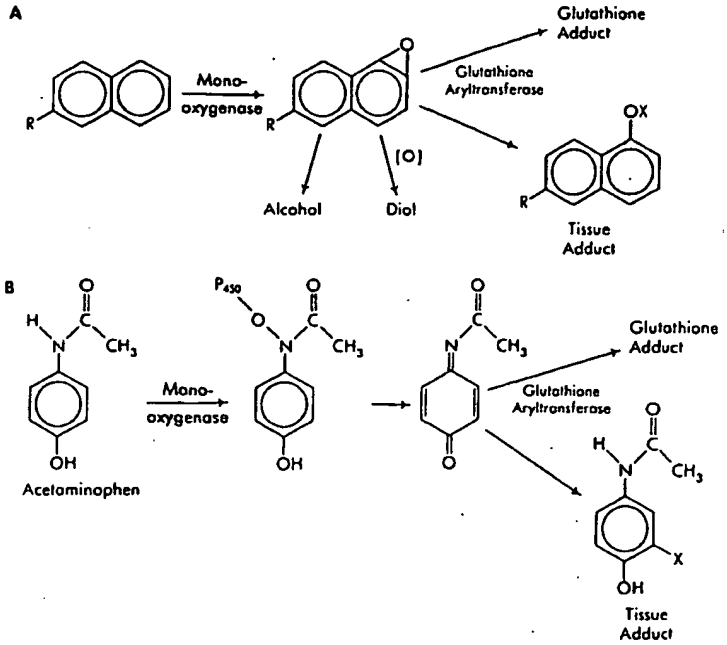

Figure 1–4. *Formation of reactive intermediates during the metabolism of drugs and environmental substances.*

A. A compound with an aromatic ring susceptible to hydroxylation may be metabolized to an arene oxide (epoxide) that can be converted spontaneously to the monoalcohol. The epoxide also can be converted enzymatically to a "diol" or can react with glutathione. The latter compound is eventually excreted as a mercapturic acid derivative. When concentrations of compounds such as glutathione are limiting, reaction can occur with macromolecular constituents of tissues.

B. Acetaminophen may be converted to a quinone type of reactive intermediate that is rapidly transformed to a mercapturate when the concentration of glutathione is not limiting. (The major route of acetaminophen metabolism is to an O-glucuronide.) X represents a tissue site of covalent reaction.

are transported by a separate system that secretes choline, histamine, and other endogenous bases.

Both carrier systems are relatively nonselective, and organic ions of similar charge compete for transport. Both transport systems can also be bidirectional, and at least some drugs are both secreted and actively reabsorbed. However, transport of most exogenous ions is predominantly secretory. The outstanding example of the bidirectional tubular transport of an endogenous organic acid is uric acid. The characteristics of tubular transport systems for organic compounds are described in detail in Chapter 30.

In the proximal and distal tubules, the nonionized forms of weak acids and bases undergo net passive reabsorption. The concentration gradient for back-diffusion is created by the reabsorption of water with Na^+ and other inorganic ions. Since the tubular cells are less permeable to the ionized forms of weak electrolytes, passive reabsorption of these substances is pH dependent. When the tubular urine is made more alkaline, weak acids are excreted more rapidly, primarily because they are more ionized and passive reabsorption is decreased. When the tubular urine is made more acidic, the excretion of weak acids is reduced. Alkalinization and acidification of the urine have the opposite effects on the excretion of weak bases. In the treatment of drug poisoning, the excretion of some drugs can be hastened by appropriate alka-

linization or acidification of the urine. Whether alteration of urine pH results in significant change in drug elimination depends upon the extent and persistence of the pH change and the contribution of pH-dependent passive reabsorption to total drug elimination. The effect is greatest for weak acids and bases with pK_a values in the range of urinary pH (5 to 8). However, alkalinization of urine can produce a fourfold to sixfold increase in excretion of a relatively strong acid such as salicylate when urinary pH is changed from 6.4 to 8.0. The fraction of nonionized drug would decrease from 1% to 0.04%.

Biliary and Fecal Excretion. Many metabolites of drugs formed in the liver are excreted into the intestinal tract in the bile. These metabolites may be excreted in the feces; more commonly, they are reabsorbed into the blood and ultimately excreted in the urine. Both organic anions, including glucuronides, and organic cations are actively transported into bile by carrier systems similar to those that transport these substances across the renal tubule. Both transport systems are nonselective, and ions of like charge may compete for transport. Steroids and related substances are transported into bile by a third carrier system. The effectiveness of the liver as an excretory organ for glucuronide conjugates is very much limited by their enzymatic hydrolysis after the bile is mixed with the contents of the small intestine, and the parent drug can be reabsorbed from the intestine. Thus, such compounds may undergo extensive biliary cycling with eventual excretion by the kidney.

Excretion by Other Routes. Excretion of drugs into sweat, saliva, and tears is quantitatively unimportant. Elimination by these routes is dependent mainly upon diffusion of the nonionized, lipid-soluble form of drugs through the epithelial cells of the glands and is pH dependent. Reabsorption of the nonionized drug from the primary secretion probably also occurs in the ducts of the glands, and active secretion of drugs across the ducts of the gland may also occur. Drugs excreted in the saliva enter the mouth, where they are usually swallowed. The concentration of some drugs in saliva parallels that in plasma. Saliva may therefore be a useful biological fluid in which to determine drug concentrations when it is difficult or inconvenient to obtain blood.

The same principles apply to excretion of drugs in breast milk. Since milk is more acidic than plasma, basic compounds may be slightly concentrated in this fluid, and the concentration of acidic compounds in the milk is lower than in plasma. Nonelectrolytes, such as ethanol and urea, readily enter breast milk and reach the same concentration as in plasma, independent of the pH of the milk. (*See* Atkinson *et al.*, 1988.)

Although excretion into hair and skin is also quantitatively unimportant, sensitive methods of detection of toxic metals in these tissues have forensic significance. Arsenic in Napoleon's hair, detected 150 years after administration, has raised interesting questions about how he died, and by whose hand. Mozart's manic behavior during the preparation of his last major work, the *Requiem*, may have been due to mercury poisoning; traces of the metal have been found in his hair.

CLINICAL PHARMACOKINETICS

A fundamental hypothesis of clinical pharmacokinetics is that a relationship exists between the pharmacological or toxic response to a drug and the concentration of the drug in a readily accessible site in the body (*e.g.*, blood). This hypothesis has been documented for many drugs (*see* Appendix II), although it is apparent for some drugs that no clear or simple relationship has been found between pharmacological effect and concentration in plasma. In most cases, as depicted in Figure 1–1, the concentration of drug in the systemic circulation will be related to the concentration of drug at its sites of action. The pharmacological effect that results may be the clinical effect desired, a toxic effect, or, in some cases, an effect unrelated to efficacy or toxicity. Clinical pharmacokinetics attempts to provide both a more quantitative relationship between dose and effect and the framework with which to interpret measurements of concentrations of drugs in biological fluids. The importance of pharmacokinetics in patient care rests on the improvement in efficacy that can be attained by attention to its principles when dosage regimens are chosen and modified.

The various physiological and pathophysiological variables that dictate adjustment of dosage in individual patients often do so as a result of modification of pharmacokinetic parameters. The three most important parameters are *clearance*, a measure of the body's ability to eliminate drug;

volume of distribution, a measure of the apparent space in the body available to contain the drug; and *bioavailability*, the fraction of drug absorbed as such into the systemic circulation. Of lesser importance are the *rates* of availability and distribution of the agent.

CLEARANCE

Clearance is the most important concept to be considered when a rational regimen for long-term drug administration is to be designed. The clinician usually wants to maintain steady-state concentrations of a drug within a known therapeutic range (*see* Appendix II). Assuming complete bioavailability, the steady state will be achieved when the rate of drug elimination equals the rate of drug administration:

$$\text{Dosing rate} = CL \cdot C_{ss} \qquad (1)$$

where CL is clearance and C_{ss} is the steady-state concentration of drug. Thus, if the desired steady-state concentration of drug in plasma or blood is known, the rate of clearance of drug by the patient will dictate the rate at which the drug should be administered.

The concept of clearance is extremely useful in clinical pharmacokinetics because clearance of a given drug is usually constant over the range of concentrations encountered clinically. This is true because systems for elimination of drugs are not usually saturated and, thus, the *absolute* rate of elimination of the drug is essentially a linear function of its concentration in plasma. A synonymous statement is that the elimination of most drugs follows first-order kinetics—a constant *fraction* of drug is eliminated per unit of time. If mechanisms for elimination of a given drug become saturated, the kinetics become zero-order—a constant *amount* of drug is eliminated per unit of time. Under such a circumstance, clearance becomes variable. Principles of drug clearance are similar to those of renal physiology, where, for example, creatinine clearance is defined as the rate of elimination of creatinine in the urine relative to its concentration in plasma. At the simplest level, clearance of a drug is the rate of elimination by all routes normalized to the concentration of drug C in some biological fluid:

$$CL = \text{Rate of elimination}/C \qquad (2)$$

It is important to note that clearance does not indicate how much drug is being removed but, rather, the volume of biological fluid such as blood or plasma that would have to be completely freed of drug to account for the elimination. Clearance is expressed as a volume per unit of time. Clearance is usually further defined as blood clearance (CL_b), plasma clearance (CL_p), or clearance based on the concentration of unbound or free drug (CL_u), depending on the concentration measured (C_b, C_p, or C_u). (For additional discussion of clearance concepts, *see* Benet *et al.*, 1984.)

Clearance by means of various organs of elimination is additive. Elimination of drug may occur as a result of processes that occur in the kidney, liver, and other organs. Division of the rate of elimination by each organ by a concentration of drug (*e.g.*, plasma concentration) will yield the respective clearance by that organ. Added together, these separate clearances will equal total systemic clearance:

$$CL_{renal} + CL_{hepatic} + CL_{other} = CL_{systemic} \qquad (3)$$

Other routes of elimination could include that in saliva or sweat, partition into the gut, and metabolism at other sites.

Total systemic clearance may be determined at steady state by using equation 1. For a single dose of a drug with complete bioavailability and first-order kinetics of elimination, total systemic clearance may be determined from mass balance and the integration of equation 2 over time.

$$CL = \text{Dose}/AUC \qquad (4)$$

where AUC is the total area under the curve that describes the concentration of drug in the systemic circulation as a function of time (from zero to infinity).

Examples. In Appendix II, the plasma clearance for cephalexin is reported as 4.3 $ml \cdot min^{-1} \cdot kg^{-1}$, with 91% of the drug excreted unchanged in the urine. For a 70-kg man, the total body clearance from plasma would be 300 ml/min, with renal clearance accounting for 91% of this elimination. In other words, the kidney is able to excrete cephalexin at a rate such that approximately 273 ml of

plasma would be freed of drug per minute. Because clearance is usually assumed to remain constant in a stable patient, the total rate of elimination of cephalexin will depend on the concentration of drug in the plasma (equation 2). Propranolol is cleared at a rate of 12 ml · min^{-1} · kg^{-1} (or 840 ml/min in a 70-kg man), almost exclusively by the liver. Thus, the liver is able to remove the amount of drug contained in 840 ml of plasma per minute. Of the drugs listed in Appendix II, one of the highest values of plasma clearance is that for labetalol—1750 ml/min; this value exceeds the rate of plasma (and blood) flow to the liver, the dominant organ for elimination of this drug. However, because labetalol partitions readily into red blood cells ($C_{rbc}/C_p = 1.8$), the amount of drug delivered to the excretory organ is considerably higher than suspected from measurement of its concentration in plasma. The relationship between plasma and blood clearance at steady state is given by:

$$\frac{CL_p}{CL_b} = \frac{C_b}{C_p} = 1 + H\left(\frac{C_{rbc}}{C_p} - 1\right) \qquad (5)$$

One may solve for labetalol clearance from blood by substituting the red blood cell to plasma concentration ratio and the average value for the hematocrit ($H = 0.45$). Clearance of labetalol, when measured in terms of its concentration in blood, is actually 1290 ml/min, a more reasonable value. Thus the plasma clearance may assume values that are not "physiological." A drug with an extremely low concentration in plasma that is concentrated in erythrocytes (*e.g.*, mecamylamine) can show a plasma clearance of tens of liters per minute. However, if the concentration in blood is used to define clearance, the maximal clearance possible is equal to the sum of blood flows to the various organs of elimination.

As mentioned, clearance of most drugs is constant over the range of concentration in plasma or blood that is encountered in clinical settings. This means that elimination is not saturated and the rate of elimination of drug is directly proportional to its concentration (equation 2). For drugs that exhibit saturable or dose-dependent elimination, clearance will vary with the concentration of drug, often according to the following equation:

$$\text{Total plasma clearance} = V_m/(K_m + C_p) \qquad (6)$$

where K_m represents the plasma concentration at which half of the maximal rate of elimination is reached (in units of mass/volume) and V_m is equal to the maximal rate of elimination (in units of mass/time). This equation is entirely analogous to the Michaelis–Menten equation for enzyme kinetics. Design of dosage regimens for such drugs is more complex (*see* below).

A further definition of clearance is useful for understanding the effects of pathological and physiological variables on drug elimination, particularly with respect to an individual organ. The rate of elimination of a drug by an individual organ can be defined in terms of the blood flow to the organ and the concentration of drug in the blood. The rate of presentation of drug to the organ is the product of blood flow (Q) and the arterial drug concentration (C_A), and the rate of exit of drug from the organ is the product of blood flow and the venous drug concentration (C_V). The difference between these rates at steady state is the rate of drug elimination:

$$\text{Rate of elimination} = Q \cdot C_A - Q \cdot C_V = Q(C_A - C_V) \qquad (7)$$

Division of equation 7 by the concentration of drug that enters the organ of elimination, C_A, yields an expression for clearance of the drug by the organ in question:

$$CL_{organ} = Q\left(\frac{C_A - C_V}{C_A}\right) = Q \cdot E \qquad (8)$$

The expression $(C_A - C_V)/C_A$ in equation 8 can be referred to as the extraction ratio for the drug (E).

Hepatic Clearance. The concepts developed in equation 8 have important implications for drugs that are eliminated by the liver. Consider a drug that is efficiently removed from the blood by hepatic processes—biotransformation and/or excretion of unchanged drug into the bile. In this instance, the concentration of drug in the blood leaving the liver will be low, the extraction ratio will approach unity, and the clearance of the drug from blood will become limited by hepatic blood flow. Drugs that are cleared efficiently by the liver (*e.g.*, drugs in Appendix II with clearances greater than 6 ml · min^{-1} · kg^{-1}, such as chlorpromazine, diltiazem, imipramine, lidocaine, morphine, and propranolol) are restricted in their rate of elimination not by intrahepatic processes but by the rate at which they can be transported in the blood to hepatic sites of elimination.

Additional complexities have also been considered. For example, the equations presented above do not account for drug binding to components of blood and tissues, nor do they permit an estimation of the intrinsic ability of the liver or kidney to eliminate a drug in the absence of limitations imposed by

blood flow. Extensions of the relationships of equation 8 to include expressions for protein binding and intrinsic clearance have been proposed for a number of models of hepatic elimination (*see* Roberts *et al.*, 1988). All of these models indicate that when the capacity of the eliminating organ to metabolize the drug is large in comparison with the rate of presentation of drug, the clearance will approximate the organ blood flow. In contrast, when the metabolic capability is small in comparison to the rate of drug presentation, the clearance will be proportional to the unbound fraction of drug in blood and the intrinsic clearance. Appreciation of these concepts allows one to understand a number of possibly puzzling experimental results. For example, enzyme induction or hepatic disease may change the rate of drug metabolism in an isolated hepatic microsomal enzyme system but not change clearance in the whole animal. For a drug with a high extraction ratio, clearance is limited by blood flow, and changes in the intrinsic clearance due to enzyme induction or hepatic disease should have little effect. Similarly, for drugs with high extraction ratios, changes in protein binding due to disease or competitive binding interactions should have little effect on clearance. In contrast, changes in intrinsic clearance and protein binding will affect the clearance of drugs with low extraction ratios but changes in blood flow should have little effect.

Renal Clearance. Renal clearance of a drug results in its appearance as such in the urine; changes in the pharmacokinetic properties of drugs due to renal disease may also be explained in terms of clearance concepts. However, the complications that relate to filtration, active secretion, and reabsorption must be considered. The rate of filtration of a drug depends on the volume of fluid that is filtered in the glomerulus and the unbound concentration of drug in plasma, since drug bound to protein is not filtered. The rate of secretion of drug by the kidney will depend on the binding of drug to the proteins involved in active transport relative to that bound to plasma proteins, the degree of saturation of these carriers, the rate of transfer of the drug across the tubular membrane, and the rate of delivery of the drug to the secretory site. The influences of changes in protein binding, blood flow, and the number of functional nephrons are analogous to the examples given above for hepatic elimination.

DISTRIBUTION

Volume of Distribution. Volume is a second fundamental parameter that is useful in discussing processes of drug disposition. The volume of distribution (V) relates the amount of drug in the body to the concentration of drug (C) in the blood or plasma, depending upon the fluid measured. This volume does not necessarily refer to an identifiable physiological volume, but merely to the fluid volume that would be required to contain all of the drug in the body at the same concentration as in the blood or plasma:

$$V = \text{Amount of drug in body}/C \qquad (9)$$

The plasma volume of a normal 70-kg man is 3 liters, blood volume is about 5.5 liters, extracellular fluid volume outside the plasma is 12 liters, and the volume of total body water is approximately 42 liters. However, many drugs exhibit volumes of distribution far in excess of these values. For example, if 500 μg of digoxin were in the body of a 70-kg subject, a plasma concentration of approximately 0.7 ng/ml would be observed. Dividing the amount of drug in the body by the plasma concentration yields a volume of distribution for digoxin of about 700 liters, or a value ten times greater than the total body volume of a 70-kg man. In fact, digoxin, which is relatively hydrophobic, distributes preferentially to muscle and adipose tissue and to its specific receptors, leaving a very small amount of drug in the plasma. For drugs that are extensively bound to plasma proteins but that are not bound to tissue components, the volume of distribution will approach that of the plasma volume. In contrast, certain drugs have high volumes of distribution even though most of the drug in the circulation is bound to albumin, because these drugs are also sequestered elsewhere.

The volume of distribution may vary widely depending on the pK_a of the drug, the degree of binding to plasma proteins, the partition coefficient of the drug in fat, the degree of binding to other tissues, and so forth. As might be expected, the volume of distribution for a given drug can change as a function of the patient's age, gender, disease, and body composition.

Several volume terms are commonly used to describe drug distribution, and they have been derived in a number of ways. The volume of distribution defined in equation 9 considers the body as a single homogeneous compartment (Figure 1–1). In this one-compartment model, all drug administration occurs directly into the central compartment and distribution of drug is instantaneous throughout volume (V). Clearance

of drug from this compartment occurs in a first-order fashion, as defined in equation 2; that is, the amount of drug eliminated per unit time depends on the amount (concentration) of drug in the body compartment. Figure 1–5, *A* and equation 10 describe the decline of plasma concentration with time for a drug introduced into this compartment.

$$C = (\text{Dose}/V) \cdot exp(-kt) \qquad (10)$$

where k is the rate constant for elimination of the drug from the compartment. This rate constant is inversely related to the half-life of the drug ($k = 0.693/t_{1/2}$).

For most drugs the idealized one-compartment model discussed above does not describe the entire time course of the plasma concentration. That is, certain tissue reservoirs can be distinguished from the central compartment, and the drug concentration appears to decay in a manner that can be described by multiple exponential terms (*see* Figure 1–5, *B*).

Rate of Drug Distribution. The multiple exponential decay observed for a drug that is eliminated from the body with first-order kinetics results from differences in the rates at which the drug equilibrates with tissue reservoirs. The rate of equilibration will depend upon the ratio of the perfusion of the tissue to the partition of drug into the tissue. In

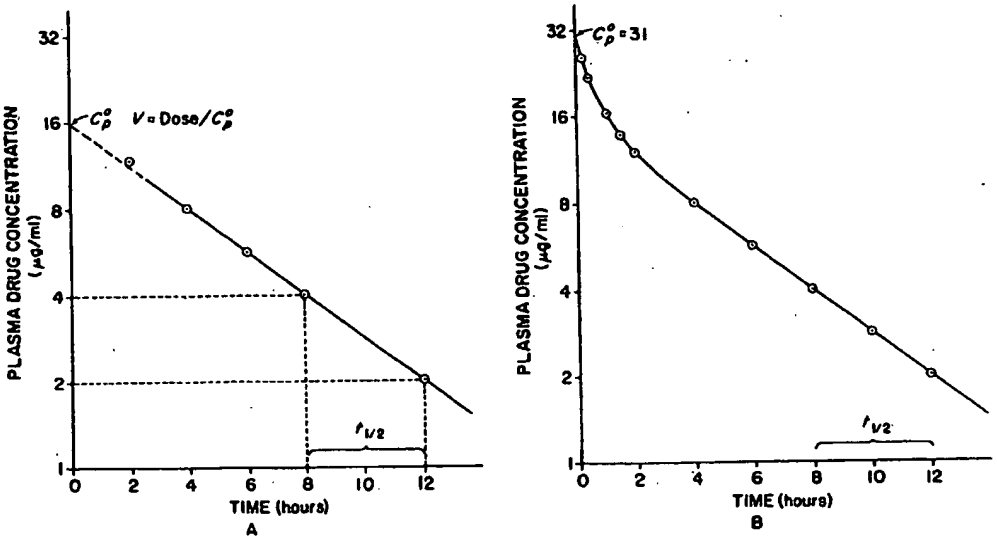

Figure 1–5. *Plasma concentration–time curves following intravenous administration of a drug (500 mg) to a 70-kg man.*

A. In this example, drug concentrations are measured in plasma 2 hours after the dose is administered. The semilogarithmic plot of plasma concentration versus time appears to indicate that the drug is eliminated from a single compartment by a first-order process (equation 10) with a half-life of 4 hours ($k = 0.693/t_{1/2} = 0.173\ hr^{-1}$). The volume of distribution (V) may be determined from the value of C_p obtained by extrapolation to $t = 0$ ($C_p^o = 16\ \mu g/ml$). Volume of distribution (equation 9) for the one-compartment model is 31.3 liters or 0.45 liter/kg ($V = \text{dose}/C_p^o$). The clearance for this drug is 92 ml/min; for a one-compartment model, $CL = k \cdot V$.

B. Sampling before 2 hours indicates that, in fact, the drug follows multiexponential kinetics. The terminal disposition half-life is 4 hours, clearance is 103 ml/min (equation 4), V_{area} is 28 liters (equation 11), and V_{ss} is 25.4 liters (equation 12). The initial or "central" distribution volume for the drug ($V_1 = \text{dose}/C_p^o$) is 16.1 liters. The example chosen indicates that multicompartment kinetics may be overlooked when sampling at early times is neglected. In this particular case, there is only a 10% error in the estimate of clearance when the multicompartment characteristics are ignored. However, for many drugs multicompartment kinetics may be observed for significant periods of time, and failure to consider the distribution phase can lead to significant errors in estimates of clearance and in predictions of the appropriate dosage.

many cases, groups of tissues with similar perfusion/partition ratios all equilibrate at essentially the same rate, such that only one apparent phase of distribution (rapid initial fall of concentration, as in Figure 1–5, *B*) is seen. It is as though the drug starts in a "central" volume, which consists of plasma and tissue reservoirs that are in rapid equilibrium with it, and distributes to a "final" volume, at which point concentrations in plasma decrease in a log-linear fashion at rate k (*see* Figure 1–5, *B*).

If the pattern or ratio of blood flows to various tissues changes within an individual or differs between individuals, rates of drug distribution to tissues will also change. However, changes in blood flow may also cause some tissues that were originally in the "central" volume to equilibrate sufficiently more slowly so as to appear only in the "final" volume. This means that central volumes will appear to vary with disease states that cause altered regional blood flow. After an intravenous bolus dose, drug concentrations in plasma may be higher in individuals with poor perfusion (*e.g.*, shock) than they would be if perfusion were better. These higher systemic concentrations may, in turn, cause higher concentrations (and greater effects) in tissues such as brain and heart whose usually high perfusion has not been reduced by the altered hemodynamic state. Thus, the effect of a drug at various sites of action can be variable, depending on perfusion of these sites.

Multicompartment Volume Terms. Two different terms have been used to describe the volume of distribution for drugs that follow multiple exponential decay. The first, designated V_{area}, is calculated as the ratio of clearance to the rate of decline of concentration during the elimination (final) phase of the logarithmic concentration versus time curve:

$$V_{area} = \frac{CL}{k} = \frac{\text{Dose}}{k \cdot AUC} \qquad (11)$$

The calculation of this parameter is straightforward, and the volume term may be determined after administration of drug by intravenous or enteral routes (where the dose used must be corrected for bioavailability). However, another multicompartment volume of distribution may be more useful, especially when the effect of disease states on pharmacokinetics is to be determined. The volume of distribution at steady state (V_{ss}) represents the volume in which a drug would appear to be distributed during steady state if the drug existed throughout that volume at the same concentration as that in the measured fluid (plasma or blood). This volume can be determined by the use of areas, as described by Benet and Galeazzi (1979):

$$V_{ss} = (\text{Dose}_{iv})(AUMC)/AUC^2 \qquad (12)$$

where $AUMC$ is the area under the first moment of the curve that describes the time course of the plasma or blood concentration, that is, the area under the curve of the product of time t and plasma or blood concentration C over the time span zero to infinity.

Although V_{area} is a convenient and easily calculated parameter, it varies when the rate constant for drug elimination changes, even when there has been no change in the distribution space. This is because the terminal rate of decline of the concentration of drug in blood or plasma depends not only on clearance but also on the rates of distribution of drug between the central and final volumes. V_{ss} does not suffer from this disadvantage (*see* Benet *et al.*, 1984).

HALF-LIFE

The half-life ($t_{1/2}$) is the time it takes for the plasma concentration or the amount of drug in the body to be reduced by 50%. For the simplest case, the one-compartment model (Figure 1–5, *A*), half-life may be determined readily and used to make decisions about drug dosage. However, as indicated in Figure 1–5, *B*, drug concentrations in plasma often follow a multiexponential pattern of decline; two or more half-life terms may thus be calculated.

In the past, the half-life that was usually reported corresponded to the terminal log-linear phase of elimination. However, as greater analytical sensitivity has been achieved, the lower concentrations measured appeared to yield longer and longer terminal half-lives. For example, a terminal half-life of 53 hours is observed for gentamicin (versus the 2-to-3-hour value in Appendix II), and biliary cycling is probably responsible for the 120-hour terminal value for indomethacin (as compared with the 2.4-hour half-life listed in Appendix II). The relevance of a particular half-life may be defined in terms of the fraction of the clearance and volume of distribution that is related to each half-life and whether plasma concentrations or amounts of drug in the body are best related to measures of response (*see* Benet, 1984). The single half-life values given for each drug in Appendix II are chosen to represent the most clinically relevant half-life.

Early studies of pharmacokinetic properties of drugs in disease were compromised by their reliance on half-life as the sole measure of alterations of drug disposition. Only recently has it been appreciated that half-life is a derived parameter that changes as a function of both clearance and volume of distribution. A useful approximate relationship between the clinically relevant half-life, clearance, and volume of distribution is given by:

$$t_{1/2} \cong 0.693 \cdot V/CL \qquad (13)$$

Clearance is the measure of the body's ability to eliminate a drug. However, the organs of elimination can only clear drug from the blood or plasma with which they are in direct contact. As clearance decreases, due to a disease process, for example, half-life would be expected to increase. However, this reciprocal relationship is exact only when the disease does not change the volume of distribution. For example, the half-life of diazepam increases with increasing age; however, it is not clearance that changes as a function of age, but the volume of distribution (Klotz *et al.*, 1975). Similarly, changes in protein binding of the drug may affect its clearance as well as its volume of distribution, leading to unpredictable changes in half-life as a function of disease. The half-life of tolbutamide, for example, decreases in patients with acute viral hepatitis, exactly the opposite from what one might expect. The disease appears to modify protein binding in both plasma and tissues, causing no change in volume of distribution but an increase in total clearance because higher concentrations of free drug are present (Williams *et al.*, 1977).

Although it can be a poor index of drug elimination, half-life does provide a good indication of the time required to reach steady state after a dosage regimen is initiated (*i.e.*, four half-lives to reach approximately 94% of a new steady state), the time for a drug to be removed from the body, and a means to estimate the appropriate dosing interval (*see* below).

Steady State. Equation 1 indicates that a steady-state concentration will eventually be achieved when a drug is administered at a constant rate. At this point, drug elimination (the product of clearance and concentration; equation 2) will equal the rate of drug availability. This concept also extends to intermittent dosage (*e.g.*, 250 mg of drug every 8 hours). During each interdose interval, the concentration of drug rises and falls. At steady state, the entire cycle is repeated identically in each interval. Equation 1 still applies for intermittent dosing, but it now describes the average drug concentration during an interdose interval. Steady-state dosing is illustrated in Figure 1–6.

Extent and Rate of Availability

Bioavailability. It is important to distinguish between the rate and extent of drug absorption and the amount that ultimately reaches the systemic circulation, as discussed above. The amount of the drug that reaches the systemic circulation can be expressed as a fraction of the dose F, which is often called bioavailability. Reasons for incomplete absorption have been discussed above. Also, as noted previously, if the drug is metabolized in the liver or excreted in bile, some of the active drug absorbed from the gastrointestinal tract will be inactivated by the liver before it can reach the general circulation and be distributed to its sites of action.

Knowing the extraction ratio (E) for a drug across the liver (*see* equation 8), it is possible to predict the maximum oral availability (F_{max}), assuming hepatic elimination follows first-order processes:

$$F_{max} = 1 - E = 1 - (CL_{hepatic}/Q_{hepatic}) \qquad (14)$$

Thus, if the hepatic blood clearance for the drug is large relative to hepatic blood flow, the extent of availability will be low when it is given orally (*e.g.*, lidocaine). This decrease in availability is a function of the physiological site from which absorption takes place, and no modification of dosage form will improve the availability under conditions of linear kinetics.

When drugs are administered by a route that is subject to first-pass loss, the equations presented previously that contain the terms *dose* or *dosing rate* (equations 1, 4, 10, and 11) must also include the bioavailability term F such that the available dose or dosing rate is used. For example, equation 1 is modified to:

$$F \cdot \text{Dosing rate} = CL \cdot C_{ss} \qquad (15)$$

Rate of Absorption. Although the rate of drug absorption does not, in general, influence the average steady-state concentration of the drug in plasma, it may still influence drug therapy. If a drug is absorbed very rapidly (*e.g.*, a dose given as an intravenous bolus) and has a small central vol-

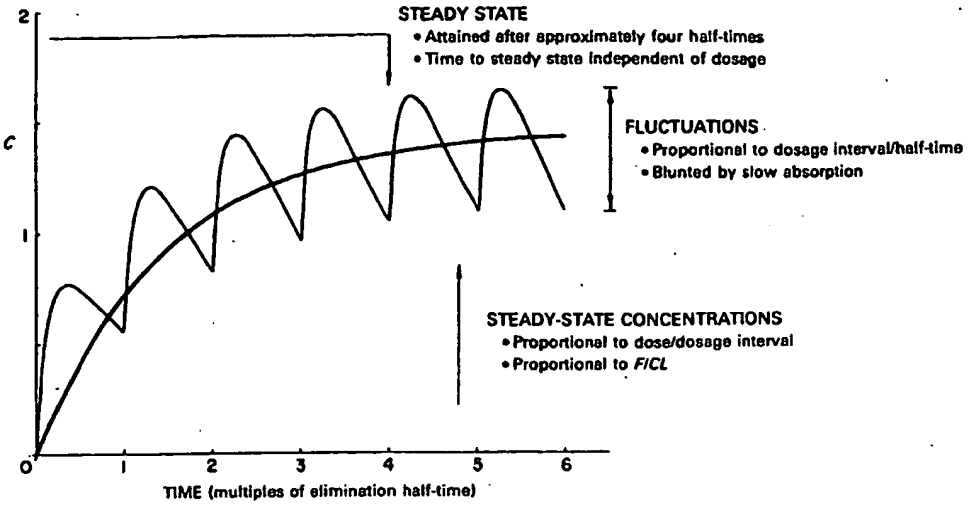

Figure 1–6. *Fundamental pharmacokinetic relationships for repeated administration of drugs.*

Light line is the pattern of drug accumulation during repeated administration of a drug at intervals equal to its elimination half-time, when drug absorption is ten times as rapid as elimination. As the relative rate of absorption increases, the concentration maxima approach 2 and the minima approach 1 during the steady state. Heavy line depicts the pattern during administration of equivalent dosage by continuous intravenous infusion. Curves are based upon the one-compartment model.

Average concentration ($\bar{C}_{ss}$) when the steady state is attained during intermittent drug administration:

$$\bar{C}_{ss} = \frac{F \cdot \text{dose}}{CL \cdot T}$$

where F = fractional bioavailability of the dose and T = dosage interval (time). By substitution of infusion rate for $F \cdot \text{dose}/T$, the formula is equivalent to equation 1 and provides the concentration maintained at steady state during continuous intravenous infusion.

ume, the concentration of drug will be high initially. It will then fall as the drug is distributed to its final (larger) volume (*see* Figure 1–5, *B*). If the same drug is absorbed more slowly (*e.g.*, by slow infusion), it will be distributed while it is being given, and peak concentrations will be lower and will occur later. A given drug may act to produce both desirable and undesirable effects at several sites in the body, and the rates of distribution of drug to these sites may not be the same. The relative intensities of these different effects of a drug may thus vary transiently when its rate of administration is changed.

NONLINEAR PHARMACOKINETICS

Nonlinearity in pharmacokinetics (*i.e.*, changes in such parameters as clearance, volume of distribution, and half-life as a function of dose or concentration of drug) is usually due to saturation of protein binding, hepatic metabolism, or active renal transport of the drug.

Saturable Protein Binding. As the molar concentration of drug increases, the unbound fraction must eventually also increase (as all binding sites become saturated). This usually occurs only when drug concentrations in plasma are in the range of tens to hundreds of micrograms per milliliter. For a drug that is metabolized by the liver with a low extraction ratio, saturation of plasma protein binding will cause both V and clearance to increase as drug concentrations increase; half-life may thus remain constant (*see* equation 13). For such a drug, C_{ss} will not increase linearly as the rate of drug administration is increased. For drugs that are cleared with high extraction ratios, C_{ss} can remain linearly proportional to the rate of drug administration. In this case, hepatic clearance would not change, and the increase in V would increase the half-time of disap-

pearance by reducing the fraction of the total drug in the body that is delivered to the liver per unit time. Most drugs fall between these two extremes, and the effects of nonlinear protein binding may be difficult to predict.

Saturable Metabolism. In this situation, the Michaelis–Menten equation (equation 6) usually describes the nonlinearity. All active processes are undoubtedly saturable, but they will appear to be linear if values of drug concentrations encountered in practice are much less than K_m. When they exceed K_m, nonlinear kinetics is observed. The major consequences of saturation of metabolism are the opposite of those for saturation of protein binding. When both conditions are present simultaneously, they may virtually cancel each others' effects, and surprisingly linear kinetics may result; this occurs over a certain range of concentrations for salicylic acid.

Saturable metabolism causes first-pass metabolism to be less than expected (higher F), and there is a greater fractional increase in C_{ss} than the corresponding fractional increase in the rate of drug administration. The latter can be seen most easily by substituting equation 6 into equation 1 and solving for the steady-state concentration:

$$C_{ss} = \frac{\text{Dosing rate} \cdot K_m}{V_m - \text{Dosing rate}} \qquad (16)$$

As the dosing rate approaches the maximal elimination rate (V_m), the denominator of equation 16 approaches zero and C_{ss} increases disproportionately. Fortunately, saturation of metabolism should have no effect on the volume of distribution; thus, as clearance decreases, the apparent half-life for elimination increases and the approach to the (disproportionate) new steady state is slow. However, the concept of "four half-lives to steady state" is not applicable for drugs with nonlinear metabolism in the usual range of clinical concentrations.

Phenytoin provides an example of a drug for which metabolism becomes saturated in the therapeutic range of concentrations (*see* Appendix II). K_m is typically near the lower end of the therapeutic range (K_m = 5 to 10 mg per liter). For some individuals, especially children, K_m may be as low as 1 mg per liter. If, for such an individual, the target concentration is 15 mg per liter and this is attained at a dosing rate of 300 mg per day, then, from equation 16, V_m equals 320 mg per day. For such a patient, a dose 10% less than optimal (*i.e.*, 270 mg per day) will produce a C_{ss} of 5 mg per liter, well below the desired value. In contrast, a dose 10% greater than optimal (330 mg per day) will exceed metabolic capacity (by 10 mg per day) and cause a long and slow but unending climb in concentration until toxicity occurs. Dosage cannot be controlled so precisely (less than 10% error). Therefore, for those patients in whom the target concentration for phenytoin is more than tenfold greater than the K_m, alternating inefficacious therapy and toxicity is almost unavoidable.

Design and Optimization of Dosage Regimens

When long-term therapy is initiated, a pharmacodynamic question must be asked: What degree of drug effect is desired and achievable? If some effect of the drug is easily measured (*e.g.*, blood pressure), it can be used to guide dosage, and a trial-and-error approach to optimal dosage is both practical and sensible. Even in this ideal case, certain quantitative issues arise, such as how often to change dosage and by how much. These can usually be settled with simple rules of thumb based on the principles discussed (*e.g.*, change dosage by no more than 50% and no more often than every three to four half-lives). Alternatively, some drugs have very little dose-related toxicity, and maximum efficacy is usually desired. For these drugs, doses well in excess of the average required will both ensure efficacy (if this is possible) and prolong drug action. Such a "maximal dose" strategy is typically used for penicillins and most β-adrenergic blocking agents.

Target Level. For some drugs, the effects are difficult to measure (or the drug is given for prophylaxis), toxicity and lack of efficacy are both potential dangers, and/or the therapeutic index is narrow. In these circumstances doses must be titrated carefully, and a target-level strategy is reasonable. A desired (target) steady-state concentration of the drug (usually in plasma) is chosen, and a dosage is computed that is expected to achieve this value. Drug concentrations are subsequently measured, and dosage is adjusted if necessary to approximate the target more closely (*see also* Chapter 4).

To apply the target-level strategy, the therapeutic objective must be defined in terms of a desirable range for the C_{ss}, often called the therapeutic range. For drugs for which this can be done, such as theophylline and digoxin, the lower limit of the therapeutic range appears to be approximately equal to the drug concentration that produces about half of the greatest possible therapeutic effect. The upper limit of the therapeutic range (for drugs with such a limit) is fixed by toxicity, not by efficacy.

In general, the upper limit of the therapeutic range is such that no more than 5 to 10% of patients will experience a toxic effect. For some drugs, this may mean that the upper limit of the range is no more than twice the lower limit. Of course, these figures can be highly variable, and some patients may benefit greatly from drug concentrations that exceed the therapeutic range while others may suffer significant toxicity at much lower values. Barring more specific information, however, the target is usually chosen as the center of the therapeutic range.

Maintenance Dose. In most clinical situations, drugs are administered in a series of repetitive doses or as a continuous infusion in order to maintain a steady-state concentration of drug in plasma within a given therapeutic range. Thus, calculation of the appropriate maintenance dosage is a primary goal. To maintain the chosen steady-state or target concentration, the rate of drug administration is adjusted such that the rate of input equals the rate of loss. This relationship was defined previously in equations 1 and 15 and is expressed here in terms of the desired target concentration:

$$\text{Dosing rate} = \text{Target} \cdot CL/F \tag{17}$$

If the clinician chooses the desired concentration of drug in plasma and knows the clearance and availability for that drug in a particular patient, the appropriate dose and dosing interval can be calculated.

Example. A steady-state plasma concentration of theophylline of 15 mg per liter is desired to relieve acute bronchial asthma in a 68-kg patient. If the patient does not smoke and is otherwise normal except for the asthmatic condition, one can use the mean clearance given in Appendix II, that is, 0.65 ml · min^{-1} · kg^{-1}. Because the drug is to be given as an intravenous infusion, $F = 1$:

$$\begin{aligned}\text{Dosing rate} &= \text{Target} \cdot CL/F \\ &= 15\ \mu\text{g/ml} \cdot 0.65\ \text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \\ &= 9.75\ \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \\ &= 40\ \text{mg/hr for a 68-kg patient}\end{aligned}$$

Since almost all intravenous preparations of theophylline are available as the ethylenediamine salt (aminophylline), which contains 85% theophylline, the infusion rate will be 47 mg per hour of aminophylline [(40 mg per hour)/(0.85)].

Dosing Interval for Intermittent Dosage. In general, marked fluctuations in drug concentrations between doses are not beneficial. If absorption and distribution were instantaneous, fluctuation of drug concentrations between doses would be governed entirely by the drug's elimination half-life. If the dosing interval (T) was chosen to be equal to the half-life, then the total fluctuation would be twofold; this is usually a tolerable variation.

Pharmacodynamic considerations modify this. If a drug is relatively nontoxic, such that concentrations many times that necessary for therapy can easily be tolerated, the maximal dose strategy can be used and the dosing interval can be much longer than the elimination half-life (for convenience). The half-life of penicillin G is less than 1 hour, but it is often given in very large doses every 6 or 12 hours.

For some drugs with a narrow therapeutic range, it may be important to estimate the maximal and minimal concentrations that will occur for a particular dosing interval. The minimal steady-state concentration $C_{ss,min}$ may be reasonably determined by the use of equation 18:

$$C_{ss,min} = \frac{F \cdot \text{dose}/V_{ss}}{1 - exp(-kT)} \cdot exp(-kT) \tag{18}$$

where k equals 0.693 divided by the clinically relevant plasma half-life and T is the dosing interval. The term $exp(-kT)$ is, in fact, the fraction of the last dose (corrected for bioavailability) that remains in the body at the end of a dosing interval.

For drugs that follow multiexponential kinetics and that are administered orally, the estimation of the maximal steady-state concentration $C_{ss,max}$ involves a complicated set of exponential constants for distribution and absorption. If these terms are ignored for multiple oral dosing, one may easily predict a maximal steady-state concentration by omitting the $exp(-kT)$ term in the numerator of equation 18 (*see* equation 19, below). Because of the approximation, the predicted maximal concentration from equation 19 will be greater than that actually observed.

Example. When the acute asthmatic attack in the patient discussed above is relieved, the clinician might want to maintain the plasma concentration of theophylline at 15 mg per liter, with oral dosage at intervals of 6, 8, or 12 hours. The correct rate of drug administration, independent of consideration of the dosing interval, is 40 mg per hour for this patient, as calculated above, since the availability of theophylline from an oral dose is 100%. Thus, the appropriate intermittent doses would be 240 mg every 6 hours, 320 mg every 8 hours, or 480 mg every 12 hours. All of these regimens would yield the same average concentration of 15 mg per liter, but different maximal and minimal concentrations would obtain. For a 12-hour dosing interval, the following maximal and minimal concentrations would be predicted:

$$C_{ss,max} = \frac{F \cdot \text{dose}/V_{ss}}{1 - exp(-kT)} = \frac{480 \text{ mg}/34 \text{ liters}}{0.65} = 22 \text{ mg/liter} \quad (19)$$

$$C_{ss,min} = C_{ss,max} \cdot exp(-kT) = (21.7 \text{ mg/liter}) \cdot (0.35) = 7.6 \text{ mg/liter} \quad (20)$$

The calculations in equations 19 and 20 were performed assuming oral doses of 480 mg every 12 hours of a drug with a half-life of 8 hours (k = 0.693/8 hr = 0.0866 hr^{-1}), a volume of distribution of 0.5 liter/kg (V_{ss} = 34 liters for a 68-kg patient), and an oral availability of 1. Since the predicted minimal concentration, 7.6 mg per liter, falls below the suggested effective concentration and the predicted maximal concentration is above that suggested to avoid toxicity (*see* Appendix II), the choice of a 12-hour dosing interval is probably inappropriate. A more appropriate choice would be 320 mg every 8 hours or 240 mg every 6 hours; for T = 6 hr, $C_{ss,max}$ = 17 mg per liter; $C_{ss,min}$ = 10 mg per liter. Of course the clinician must balance the problem of compliance with regimens that involve frequent dosage against the problem of periods when the patient may be subjected to concentrations of the drug that could be too high or too low.

Loading Dose. The "loading dose" is one or a series of doses that may be given at the onset of therapy with the aim of achieving the target concentration rapidly. The appropriate magnitude for the loading dose is:

$$\text{Loading dose} = \text{Target } C_p \cdot V_{ss}/F \quad (21)$$

A loading dose may be desirable if the time required to attain steady state by the administration of drug at a constant rate (four elimination half-lives) is long relative to the temporal demands of the condition being treated. For example, the half-life of lidocaine is usually more than 1 hour. Arrhythmias encountered after myocardial infarction may obviously be life threatening, and one cannot wait 4 to 6 hours to achieve a therapeutic concentration of lidocaine by infusion of the drug at the rate required to maintain this concentration. Hence, use of a loading dose of lidocaine in the coronary care unit is standard.

The use of a loading dose also has significant disadvantages. First, the particularly sensitive individual may be exposed abruptly to a toxic concentration of a drug. Moreover, if the drug involved has a long half-life, it will take a long time for the concentration to fall if the level achieved was excessive. Loading doses tend to be large, and they are often given parenterally and rapidly; this can be particularly dangerous if toxic effects occur as a result of actions of the drug at sites that are in rapid equilibrium with plasma.

Individualizing Dosage. To design a rational dosage regimen, the clinician must know F, CL, V_{ss}, and $t_{1/2}$, and have some knowledge about rates of absorption and distribution of the drug. Moreover, one must judge what variations in these parameters might be expected in a particular patient. Usual values for the important parameters and appropriate adjustments that may be necessitated by disease or other factors are presented in Appendix II. There is, however, unpredictable variation between normal individuals; for many drugs, one standard deviation in the values observed for F, CL, and V_{ss} is about 20%, 50%, and 30%, respectively. This means that 95% of the time the C_{ss} that is achieved will be between 35% and 270% of the target; this is an unacceptably wide range for a drug with a low therapeutic index. If values of C_p are measured, one can estimate values of F, CL, and V_{ss} directly, and this permits more precise adjustment of a dosage regimen. Such measurement and adjustment are appropriate for many drugs with low therapeutic indices (*e.g.*, cardiac glycosides, antiarrhythmic agents, anticonvulsants, theophylline, and others).

THERAPEUTIC DRUG MONITORING

The major use of measured concentrations of drugs (at steady state) is to refine the estimate of CL/F for the patient being treated (using equation 15 as rearranged below):

$$CL/F \text{ (patient)} = \text{Dosing rate}/C_{ss} \text{ (measured)} \quad (22)$$

The new estimate of CL/F can be used in equation 17 to adjust the maintenance dose to achieve the desired target concentration.

Certain practical details and pitfalls related to therapeutic drug monitoring should be kept in mind. The first of these concerns the time of sam-

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pling for measurement of the drug concentration. If intermittent dosing is used, when during a dosing interval should samples be taken? It is necessary to distinguish between two possible uses of measured drug concentrations in order to understand the possible answers. A concentration of drug measured in a sample taken at virtually any time during the dosing interval will provide information that may aid in the assessment of drug toxicity. This is one type of therapeutic drug monitoring. It should be stressed, however, that such use of a measured concentration of drug is fraught with difficulties because of interindividual variability in sensitivity to the drug. When there is a question of toxicity, the drug concentration can be no more than just one of many items that serve to inform the clinician.

Changes in the effects of drugs may be delayed relative to changes in plasma concentration because of a slow rate of distribution or pharmacodynamic factors. Concentrations of digoxin, for example, regularly exceed 2 ng/ml (a potentially toxic value) shortly after an oral dose, yet these peak concentrations do not cause toxicity; indeed, they occur well before peak effects. Thus, concentrations of drugs in samples obtained shortly after administration can be uninformative or even misleading.

When concentrations of drugs are used for purposes of adjusting dosage regimens, samples obtained shortly after administration of a dose are almost invariably misleading. The point of sampling during supposed steady state is to modify one's estimate of *CL/F* and thus one's choice of dosage. Early postabsorptive concentrations do not reflect clearance; they are determined primarily by the rate of absorption, the central (rather than the steady-state) volume of distribution, and the rate of distribution, all of which are pharmacokinetic features of virtually no relevance in choosing the long-term maintenance dosage. When the goal of measurement is adjustment of dosage, the sample should be taken well after the previous dose—as a rule of thumb just before the next planned dose, when the concentration is at its minimum. There is an exception to this approach: some drugs are nearly completely eliminated between doses and act only during the initial portion of each dosing interval. If, for such drugs, it is questionable whether efficacious concentrations are being achieved, a sample taken shortly after a dose may be helpful. Yet, if another concern is that low clearance (as in renal failure) may cause accumulation of drug, concentrations measured just before the next dose will reveal such accumulation and are considerably more useful for this purpose than is knowledge of the maximal concentration. For such drugs, determination of both maximal and minimal concentrations is thus recommended.

A second important aspect of the timing of sampling is its relationship to the beginning of the maintenance dosage regimen. When constant dosage is given, steady state is reached only after four half-lives have passed. If a sample is obtained too soon after dosage is begun, it will not accurately reflect clearance. Yet, for toxic drugs, if one waits until steady state is ensured, the damage may have been done. Some simple guidelines can be offered. When it is important to maintain careful control of concentrations, one may take the first sample after two half-lives (as calculated and expected for the patient), assuming no loading dose has been given. If the concentration already exceeds 90% of the eventual expected mean steady-state concentration, the dosage rate should be halved, another sample obtained in another two (supposed) half-lives, and the dosage halved again if this sample exceeds the target. If the first concentration is not too high, one proceeds with the initial rate of dosage; even if the concentration is lower than expected, one can usually await the attainment of steady state in another two estimated half-lives and then proceed to adjust dosage as described above.

If dosage is intermittent, there is a third concern with the time at which samples are obtained for determination of drug concentrations. If the sample has been obtained just prior to the next dose, as recommended, concentration will be a minimal value, not the mean. However, as discussed above, the estimated mean concentration may be calculated by using equation 15.

If a drug follows first-order kinetics, the average, minimum, and maximum concentrations at steady state are linearly related to dose and dosing rate (*see* equations 15, 18, and 19). Therefore, the ratio between the measured and the desired concentrations can be used to adjust the dose:

$$\frac{C_{ss}(\text{measured})}{C_{ss}(\text{desired})} = \frac{\text{Dose(previous)}}{\text{Dose(new)}} \qquad (23)$$

Finally, for some drugs that are particularly difficult to manage, computer programs may be useful for the design of dosage regimens. Such programs, which take into account measured drug concentrations and individual factors such as those listed in Appendix II, are becoming increasingly available (see Sheiner *et al.*, 1972; Vozeh and Steimer, 1985).

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